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13. ABSTRACT *(Maximum 200 words)*

Using the procedures described in this report, we were able to work sequentially or simultaneously on development, validation and characterization of assays for WR 238,605 (and its stereoisomers), halofantrine (and its metabolite and their stereoisomers), WR 6026 (and its metabolites), mefloquine (and its metabolite), artelinic acid, *p*-aminoheptanophenone (and related compounds), gentamicin and paromomycin, pyridostigmine, WR 242511, chloroquine (and its metabolites), WR 243,251, quinine and doxycycline. Work on routine analyses of biological specimens during this period was performed for studies that required determination of concentrations of WR 238,605 (and its stereoisomers), halofantrine (and its metabolite and their stereoisomers), WR 6026 (and its metabolites), mefloquine, *p*-aminoheptanophenone (and related compounds), primaquine, gentamicin and paromomycin, chloroquine (and its metabolites), quinine, and doxycycline. We worked on demonstrating sensitivity, specificity, linearity, lack of interferences, accuracy, and reproducibility of the analytical method, describing the extent of recovery for the method, and reporting on the stability of compounds of interest in specimens during storage and drug analysis to provide documentation in support of Investigational New Drug (IND) submissions to the Food and Drug Administration (FDA).

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FOREWORD

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PI - Signature 9/11/98 Date

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INTRODUCTION

This report describes technical work accomplished and information gained in performance of contract number DAMD17-97-C-7058, titled "Analysis of Investigational Drugs in Biological Fluids - Method Development and Analysis of Pre-Clinical and Clinical Samples," for the US Army Medical Research and Development Command (USAMRDC).

For many years our research group has been actively involved in the development of analytical methods to assay for drug substances in biological fluids for pharmacokinetic, bioavailability, drug metabolism and drug monitoring studies. This report describes the approach we took to develop sensitive (picograms per milliliter of biological matrix), specific and quantitative analytical methods to support pharmacokinetic and bioavailability studies of candidate chemical warfare antidotes, antiparasitic drugs, radioprotectants and anti-infectious disease drugs.

In addition, routine analyses of biological specimens to support pharmacokinetic and bioavailability studies as part of preclinical and clinical investigations undertaken for the purpose of new drug development were performed as a significant adjunct to method development objectives. Within our routine analysis laboratory, we maintain the capability to assay up to 3,000 samples per year for this contract.

There are many reasons for the U.S. military to develop various new drugs to protect or to treat soldiers confronted with the hazards of the modern battlefield. Like any pharmaceutical company, however, the military has to provide documentation in support of Investigational New Drug (IND) submissions to the Food and Drug Administration (FDA). Therefore, a great deal of work involving animal studies, preclinical and clinical trials, toxicity, metabolism and formulations must be carried out before a drug can be tried in the field. All of these studies depend on the adequacy of the analytical method for the particular compound. The route of administration and the dosage form are not necessarily the same in the field as in the clinic. For example, pyridostigmine is given prophylactically in the field, but the dose and route of administration are different for the treatment of myasthenia gravis or in anesthesiology.[1] Since military personnel are constantly involved in areas where they can be infected by parasites, including tropical or subtropical zones with drug-resistant forms, the U.S. Army needs to organize programs so that highly active and more effective new drugs can be discovered. These types of programs are generally ignored by private industry due to limited markets and profits.

This contract has offered us an interesting and stimulating challenge to utilize and extend our considerable capabilities to conduct method development and routine analysis in support of pharmacokinetic and bioavailability studies. Our participation in this contract was possible by virtue of the experience and expertise of our staff in the area of pharmacokinetics, which requires assurance of extensive and rigorous internal and external analytical quality. As a result of our extensive involvement in these analytical programs, the staff members

working on this project are the best in the field and have acquired a broad range of experience in the analysis of organic compounds in diverse media.

Nature Of Problem

Using the experimental procedures described in this report, we maintain the capability to complete projects on up to one new compound (for which no method is described in the literature) and up to two compounds (for which methods are described in the literature) per year in terms of method development, validation, and characterization. We demonstrate sensitivity, specificity, linearity, lack of interferences, accuracy, and reproducibility of the analytical method, describe the extent of recovery for the method, and report on the stability of compounds of interest in specimens during storage and drug analysis. Validation of sensitive and specific analytical methods follow procedures described in the Analytical Section Procedural Manual, Procedure 2D-3.5, "Procedure for Validation" and earlier versions. Methods developed are such that a single technician can complete at least 15 clinical samples in one day. These methods are robust and portable enough to be transported to other laboratories. Within our routine analysis laboratory, we maintain the capability to assay up to 3,000 samples per year. Routine sample analysis will be performed in accordance with applicable procedures described in the Analytical Section Procedural Manual, Procedure 2D-4.5. "HPLC Run Setup" and Procedure 2D-10.3. "LC/MS/MS Run Setup" and earlier versions. We have sufficient equipment and personnel to develop several candidate agents simultaneously and to be able to respond to changing priorities. We prepare and submit required reports in accordance with the contracted schedule.

Background Of Previous Work

Studies conducted over the 13 years prior to contract DAMD17-97-C-7058 under previous contracts including DAMD17-92-C-2028, DAMD17-86-C-6150, DAMD17-85-D-0008, and DAMD17-83-C-3004 are listed in Tables 1 (study reports) and 2 (routine analyses reports).

TABLE 1: PREVIOUS STUDY REPORTS

Report No.	Report Date	Report Title	Test Article	Test System	Lower Limit of Quantitation
01	8/26/83	Analytical Procedure for the determination of WR 6026 in Plasma	WR 6026 WR211,789•2HCl WR 6026 WR211,789•2HCl	Plasma Plasma Blood Blood	6.44 ng/ml 8.00 ng/ml 6.44 ng/ml 8.00 ng/ml
03	1/22/85	High Pressure Liquid Chromatography (HPLC) of Pyridostigmine in Plasma	Pyridostigmine	Plasma	1.4 ng/ml
04	8/23/85	Ion-Paired Liquid Chromatographic Method for the Analysis of Halofantrine (WR 171,669) and its Putative Metabolite, WR 178,460, in Blood and Plasma	halofantrine WR 178,460 halofantrine WR 178,460	Plasma Plasma Blood Blood	0.900 ng/ml 1.40 ng/ml 0.900 ng/ml 1.40 ng/ml
05	7/21/86	High Pressure Liquid Chromatography (HPLC) of Pyridostigmine in Plasma Using Silica Gel Column and an Aqueous Mobile Phase	Pyridostigmine	Plasma	1.39 ng/ml
06	1/8/88	High Pressure Liquid Chromatography (HPLC) of Mefloquine in Plasma	Mefloquine	Plasma	10.0 ng/ml
07	1/12/88	High Pressure Liquid Chromatography (HPLC) of Pyridostigmine in Urine	Pyridostigmine	Urine	13.7 ng/ml
08	9/23/88	High Pressure Liquid Chromatography (HPLC) of Physostigmine in Plasma with Ultraviolet Detection	Physostigmine	Plasma	1 ng/ml
09	9/12/88	Quantitation of Physostigmine & Eseroline in Plasma by HPLC with Fluorescence Detection	Physostigmine eseroline	Plasma Plasma	0.1 ng/ml 0.1 ng/ml
10	9/14/89	Quantitation of WR 6026 (Free Base) in Plasma & Blood by HPLC	WR 6026 WR 6026	Plasma Blood	0.980 ng/ml
11	9/28/89	Quantitation of WR 2721 in Plasma by HPLC with Electrochemical Detection	WR 2721	Plasma	0.100 µg/ml

TABLE 1: PREVIOUS STUDY REPORTS

Report No.	Report Date	Report Title	Test Article	Test System	Lower Limit of Quantitation
12	11/14/89	Quantitation of WR 3689 in Plasma by HPLC with Electrochemical Detection	WR 3689	Plasma	0.0990 µg/ml
13	11/17/89	Quantitation of WR 238605 by HPLC	WR 238,605 WR 238,605	Plasma Blood	0.815 ng/ml 1.91 ng/ml
13	10/28/94 final report	Supplement I: Quantitation of WR 238605 as Free Base in Rat Plasma by HPLC and Fluorescence Detection	WR 238,605	Rat Plasma	2.00 ng/ml
13	4/11/94 in revision	Supplement II: Quantitation of WR 238605 as Free Base in Dog Plasma by HPLC and Fluorescence Detection	WR 238,605	Dog Plasma	1.00 ng/ml
14	8/29/89	Quantitation of Mefloquine (f.b.) in Plasma by HPLC, Extract. Meth	Mefloquine	Plasma	8.00 ng/ml
15	12/19/90	Quantitation of Ribavirin and WR 249,992 (f. b.) in Plasma by HPLC with C18 Bonded Silica Gel Columns and Acidic Aqueous Mobile Phases	Ribavirin WR 249,992	Plasma Plasma	20 ng/ml 10 ng/ml
16	Canceled	β-arteether project canceled	WR 255663		
17A	4/25/90 final	Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Plasma and Blood by HPLC with a Silica Gel Column and an Aqueous Mobile Phase	halofantrine WR 178,460 halofantrine WR 178,460	Human Plasma Plasma Blood Blood	0.960 ng/ml 0.928 ng/ml 0.960 ng/ml 0.928 ng/ml
17B	12/13/95 final as amended 4/26/96	Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Plasma and Blood by HPLC with a Silica Gel Column and an Aqueous Mobile Phase	halofantrine WR 178,460 halofantrine WR 178,460	Human Plasma Plasma Blood Blood	2 ng/ml 2 ng/ml 0.960 ng/ml 0.928 ng/ml
17B	11/1/96 in review	Supplement I: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Perfusate by Precipitation and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat perfusate	0.520 µg/ml 0.510 µg/ml

TABLE 1: PREVIOUS STUDY REPORTS

Report No.	Report Date	Report Title	Test Article	Test System	Lower Limit of Quantitation
17B	11/25/96 in review	Supplement II: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Perfusate by Extraction and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat perfusate	10.4 ng/ml 10.2 ng/ml
17B	12/3/96 in review	Supplement III: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Bile by Precipitation and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat bile	0.416 µg/ml 0.408 µg/ml
17B	12/5/96 in review	Supplement IV: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Bile by Extraction and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat bile	20.4 ng/ml
17B	1/28/97 in review	Supplement V: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Liver by Precipitation and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat liver	0.540 µg/ml 0.540 µg/ml
18	Status report: 7/31/91	Quantitation of WR 6026 and WR 211,789 (WR 6026 Metabolite) in Plasma and Blood by HPLC with a Silica Gel Column and an Aqueous Mobile Phase	WR 6026 WR 211789	Plasma Blood	0.980 ng/ml 1.21 ng/ml
19	Status report: 1/14/92	Tentative title: Quantitation of Mefloquine and its Metabolite, WR 160972 in Biological Fluids	mefloquine WR 160972	plasma blood	7.36 ng/ml -
20	7/27/94 final report	Quantitation of Artelinic acid in Plasma by HPLC with a C18 Bonded Column	Artelinic Acid	human plasma	4.96 ng/ml
21	Validation complete	Tentative title: Quantitation of *p*-Aminoheptanophenone, *p*-Aminooctanophenone, and *p*-Aminopropiophenone in Dog Plasma by HPLC	PAHP PAPP PAOP	dog plasma	4.08 ng/ml 4.04 ng/ml 4.16 ng/ml

TABLE 1: PREVIOUS STUDY REPORTS

Report No.	Report Date	Report Title	Test Article	Test System	Lower Limit of Quantitation
22	7/18/94 in revision	Quantitation of WR 6026, WR 211,789, and WR 254,421 (as Free Bases) in Human Urine By HPLC	WR 6026 WR 211,789 WR 254,421	human urine	5.17 ng/ml 5.09 ng/ml 45.4 ng/ml
23	4/29/96 final report	Quantitation of Primaquine (Free Base) and its Carboxylated Metabolite in Human Plasma by HPLC and Ultraviolet Detection	Primaquine WR 249725	human plasma	28.5 ng/ml 20.0 ng/ml
24	1/7/97 in review	Quantitation of Paromomycin and Gentamicin in Human and Rat Plasma by HPLC	Gentamicin Paromomycin Gentamicin Paromomycin	human plasma rat plasma	0.1 µg/ml 0.1 µg/ml 0.1 µg/ml 0.1 µg/ml
25	11/22/95 final report as amended 3/29/96	Quantitation of Pyridostigmine (as Free Base) in Human Plasma By HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Pyridostigmine	human plasma	1.53 ng/ml
26	12/12/96 final report	Quantitation of WR 242511 (as Free Base) in Human and Dog Plasma By HPLC with a Silica Gel Column and an Aqueous Mobile Phase	WR 242511 WR 242511	human plasma dog plasma	4.00 ng/ml 4.00 ng/ml
27	in develop-ment	Tentative title: Quantitation of WR 238605 R&S Separation in Human Plasma by HPLC	WR 238605	human plasma	
28	In develop-ment	Tentative title: Quantitation of R&S Isomers of Halofantrine and WR 178,460 in Human Plasma by HPLC	Halofantrine WR 178,460	human plasma	
29	In validation	Tentative title: Quantitation of Chloroquine and Desethyl-chloroquine in Human Plasma by LC/MS/MS	Chloroquine Desethyl-chloroquine	human plasma	
30	In validation	Tentative title: Quantitation of WR 243251 in Human Plasma by LC/MS/MS	WR 243251	human plasma	1 to 5 ng/ml

TABLE 1: PREVIOUS STUDY REPORTS

Report No.	Report Date	Report Title	Test Article	Test System	Lower Limit of Quantitation
31	In validation	Tentative title: Quantitation of WR 238,605, Mefloquine, Chloroquine, Quinine and Doxycycline in Dog Plasma by LC/MS/MS	WR 238605 Mefloquine Chloroquine Quinine Doxycycline	dog plasma	
32	In validation	Tentative title: Quantitation of WR 238,605 in Human Plasma by LC/MS/MS	WR 238605	human plasma	
33	In validation	Tentative title: Quantitation of Halofantrine and WR 178,460 in Human Plasma by LC/MS/MS	Halofantrine WR 178,460	human plasma	

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis of Halofantrine Plasma Samples Obtained from Protocol Titled "The Relative Bioavailability of Three Oral Formulations of Halofantrine Hydrochloride"	10/23/87	Halofantrine WR 178,460	plasma plasma	971 971	AY 86-1D
Phase III Comparative Clinical Trial of 4 Regimens of Halofantrine and Chloroquine in Treatment of P. falciparum Malaria	6/27/90	Halofantrine WR 178,460 Halofantrine WR 178,460	plasma plasma blood blood	470 470 468 468	Hal/BP 89-7
Routine Analysis for Protocol Titled "Pharmacokinetics of Intravenous Halofantrine HCl"	12/18/90	Halofantrine WR 178,460 Halofantrine WR 178,460	plasma plasma blood blood sol'ns	434 434 429 429 20	Hal/PB 90-5
Routine Analysis for Halofantrine and WR 178,460 (as Free Bases) of Plasma Samples Obtained under the Protocol Titled "52-Week Chronic Oral Toxicity Study of WR 171,669 HCl (Halofantrine Hydrochloride) in Dogs"and "Analysis of Blood and Plasma to Verify in vitro Metabolism of Halofantrine and Partition of Halofantrine and WR 178,460"	7/16/91	Halofantrine WR 178,460 Halofantrine WR 178,460	plasma plasma blood blood	83 83 48 48	Hal/P 91-1&2
Routine Analysis of Plasma and Blood Samples for the Protocol Titled 'Disposition Kinetics of IV Desbutyl Halofantrine and the Effects of Gastric pH on the Bioavailability of Halofantrine-HCl'	2/4/92	Halofantrine WR 178,460 Halofantrine WR 178,460	plasma plasma blood blood dosing sol'ns	756 756 754 754 18	Hal/BP 91-3

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for Halofantrine and WR 178,460 (as Free Bases) of Plasma Samples Obtained under the Protocol Titled "Combined Chronic Toxicity and Oncogenicity Study of WR 171,669•HCl (Halofantrine Hydrochloride) in Rats"	9/23/91	Halofantrine WR 178,460	plasma plasma	118 118	Hal/P 91-4
Routine Analysis for Halofantrine and WR 178,460 (free bases) in Blood Samples Obtained for the Protocol Titled "Efficacy of Halofantrine and Mefloquine in the Treatment of Falciparum Malaria"	1/21/92	Halofantrine WR 178,460	blood blood	107 107	Hal/B 91-5
Routine Analysis of Mefloquine Plasma Samples obtained from Six Clinical Protocols from Thailand	2/25/88	Mefloquine	plasma	781	Mef/P 87-1B
Routine Analysis of Plasma Samples from Thailand for Mefloquine Concentrations	12/7/88	Mefloquine	plasma	388	Mef/P 88-11
Routine Analysis of Blood Samples for Mefloquine (Free Base) Concentrations	2/12/91	Mefloquine	blood	18	Mef/B 90-3
Routine Analysis of Physostig-mine Plasma Samples from the Protocol Titled "Bioavail-ability and Pharmacokinetic Study of Physostigmine (WR 006570) in Beagle Dogs"	8/26/88	Physostigmine Eseroline	plasma plasma	198 198	Phy/P 88-5
Routine Analysis of Physostigmine Plasma Samples from the Protocol Titled "Bioavailability and Pharmacokinetic Study of Physostigmine (WR 006570 AM) in Rhesus Macaques"	9/15/88	Physostigmine Eseroline	plasma plasma	196 196	Phy/P 88-6
Pilot Study - Analysis of Rat Plasma	9/14/88	Physostigmine Eseroline	plasma plasma	45 45	Phy/rP 88-8

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Pilot Study - Analysis of Rat Perfusate	9/14/88	Physostigmine	perfus	37	Phy/rPr, pilot 88-9
Pilot Study - Analysis of Monkey Plasma	5/5/88	Physostigmine Eseroline	plasma plasma	8 8	Phy/mP, pilot 88-10
Routine Analysis of Physostigmine (free base) and Eseroline (free base) Rat Plasma Bile, and Tube Binding Samples for Samples Obtained from WRAIR	1/18/90	Physostigmine Eseroline Physostigmine Eseroline	plasma plasma bile etc bile etc	92 92 20 20	Phy/rP, pilot 89-6
Pyridostigmine in plasma (Israel)	5/14/86	Pyridostigmine	plasma	427	PY 85-4
"Pyridostigmine in plasma" (PY85-6-2 and PY85-6-3 combined) (Johns Hopkins, Millers)	7/3/86	Pyridostigmine	plasma	32	PY 85-6-4
Routine Analysis of Pyridostigmine Plasma Samples from Battelle Laboratories-MREF Protocol 27 (Battelle)	7/9/86	Pyridostigmine Pyridostigmine	plasmap lasma,bl ind	648 22	PY 85-2-3
Routine Analysis of Pyridostigmine Plasma Samples Obtained from Protocol Titled "Pharmacokinetics of Orally Administered Pyridostigmine and Comparative Bioavailability of Liquid and Tablet Formulations" (Subjects 1-30)	12/3/86	Pyridostigmine Pyridostigmine	plasma dose sol	1698 12	PY 85-1
"Pyridostigmine in plasma (Johns Hopkins,Sub.1-24)"	1/12/87	Pyridostigmine pyridostigmine	plasma infusate	969 23	PY 85-6-5
"Pyridostigmine in plasma (Johns Hopkins,Sub.1-24)"	3/12/87	Pyridostigmine Pyridostigmine	plasma dose sol	1102 27	PY 85-6-6B

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis of Pyridostigmine Plasma Samples obtained from Protocol Titled "Development of a Primate Model for Evaluating Efficacy of Treatment Regimens Against Nerve Agent Poisoning:Part I: Pharmacokinetics of Pralidoxime Chloride, Atropine Sulfate, and Pyridostigmine Bromide" (PY85-3-1 through PY85-3-5 combined)	5/29/87	Pyridostigmine	plasma, monkey	439	PY 85-3-6B
Battelle Rat Study Pyridostigmine in plasma (revised letter report)	7/28/87	Pyridostigmine	plasma, rat	102	none none
Battelle Dosing Sol'ns Pyridostigmine in plasma (revised letter report)	7/28/87	Pyridostigmine	dose sol	92	none none
Routine Analysis of Pyridostigmine Plasma Samples Obtained from Protocol Titled "14 day pilot dose range oral toxicity study in dogs" (Battelle)	7/30/87	Pyridostigmine Pyridostigmine	plasma dose sol	152 2	PY 85-2-2B
Pyridostigmine in plasma (Huntingdon dog)	9/30/87	Pyridostigmine	plasmadog	336	PY 85-5-3C
Routine Analysis of Pyridostigmine Plasma Samples obtained from Protocol titled "Comparative Bioavailability Studies of Pyridostigmine Bromide in Male Beagle Dogs " (31 July 1985) (Huntingdon dog)	10/7/87	Pyridostigmine	plasma, dog	324	PY 85-5C

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis of Pyridostigmine Urine Samples from Protocol Titled "Bioavailability of Oral Pyridostigmine and Inhibition of Red Blood Cell Acetylcholinesterase by Oral and Intravenous Pyridostigmine"	2/3/88	Pyridostigmine	urine	110	Pyr/U 86-3B (renamed from AY86-3)
Routine Analysis of Pyridostigmine Plasma and Urine Samples from Protocol Titled "Pharmacokinetics and Pharmacodynamics of Sustained, Low-dose, Intravenous Infusions of Pyridostigmine"	2/24/88	Pyridostigmine Pyridostigmine Pyridostigmine	plasma urine infusate	498 72 24	Pyr/PU 87-2B
Routine Analysis of Pyridostigmine Plasma Samples from the Protocol titled "Comparative Oral Bioavailability Studies of Two Wax Matrix Formulations of Pyridostig- mine Bromide in Male Beagle Dogs"	3/29/88	Pyridostigmine	plasma	341	Pyr/P 88-1
Routine Analysis of Pyridostigmine Plasma Samples from the Protocol titled "Safety, Tolerance, Pharmacokinetics and Pharmaco- dynamics of Single Oral Doses of Sustained Release Pyridostigmine in Healthy Men," dated 9/18/87	8/3/88	Pyridostigmine	plasma	558	Pyr/P 88-2
"Routine Analysis of Pyridostigmine Plasma Samples from the Protocol titled ""Safety, Tolerance, Pharmacokinetics and Pharmaco- dynamics of Single Oral Doses of Sustained Release Pyridostigmine in Healthy Men,"" dated Sept. 30, 1987"	8/2/88	Pyridostigmine	plasma	476	Pyr/P 88-3

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for Protocol Titled "Safety, Tolerance, Pharmacokinetics and Pharmaco- dynamics of Single Oral Doses of Pyridostigmine Administered by an Osmotic-Delivery Module (osmetr) compared to Pyridostigmine Syrup in Healthy Men"	5/12/89	Pyridostigmine	plasma	374	Pyr/P 89-2
Routine Analysis for protocol titled "Safety, Tolerance, Pharmacokinetics and Pharmaco- dynamics of Single Oral Doses of a Commercial Formulation of Sustained-Release Pyridostigmine in Healthy Men."	5/16/89	Pyridostigmine	plasma	120	Pyr/P 89-3
Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Intravenous Pyridostigmine and Oral Doses of Standard and Sustained-Release Pyridostigmine in Healthy Men & the Influence of Food on Oral Pyridostigmine Pharmacokinetics	11/13/90	Pyridostigmine	plasma	1250	Pyr/P 89-8
Routine Analysis for Protocol Titled "Effect of chronic pyridostigmine administration on heavy exercise in hot environments"	9/11/90	Pyridostigmine	plasma	37	Pyr/P 90-2
Routine Analysis for Protocol Titled "Effects of Pyridostigmine Pretreatment on Physiological Responses to Heat & Moderate-to Intense Exercise"	2/20/91	Pyridostigmine	plasma	142	Pyr/P 90-4
Routine Analysis for protocol titled "Simultaneous Modeling of WR238605 Succinate Pharmacokinetics and Methhemoglobin Pharmacodynamics in the Beagle Dog"	4/13/89	WR 238605 WR 238605	plasma blood	62 62	WR5/BP, 89-1 pilot

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for protocol titled "Simultaneous Modeling of WR238605 Succinate Pharmacokinetics and Methhemoglobin Pharmacodynamics in the Beagle Dog"	6/1/89	WR 238605 WR 238605	plasma blood	88 88	WR5/BP, 89-4 pilot
Routine Analysis for protocol titled "Simultaneous Modeling of WR238605 Succinate Pharmacokinetics and Methhemoglobin Pharmacodynamics in the Beagle Dog"	8/25/89	WR 238605 WR 238605	plasma blood	240 240	WR5/BP 89-5
Routine Analysis of WR 6026 Plasma Samples Obtained from Clinical Protocol Titled "Single-Dose Absorption and Pharmacokinetics of WR 6026 Hydrochloride in Healthy Subjects"	6/24/87	WR 6026	plasma	192	AY 86-2D
Routine Analysis of Blood Samples from the Protocol Titled "Multiple-Dose Pharmacokinetics, Safety and Tolerance of WR 6026 Hydrochloride in Healthy Subjects"	4/21/89	WR 6026	blood	571	Wr6/B 88-7
Routine Analysis for WR 6026 and WR 211,789 (as Free Bases) of Plasma Samples Obtained from WRAIR - Preliminary Report	2/13/91	WR 6026 WR 211789	plasma plasma	13 13	Wr6/PB 90-6
Routine Analysis for Halofantrine and WR 178,460 (as free bases) of Plasma Samples Obtained for the Initial Year of the Protocol Titled "Combined Chronic Toxicity and Oncogenicity Study of WR-171,669 HCl (Halofantrine Hydrochloride) in Rats"	3/31/93 final report	halofantrine WR 178,460	rat plasma	118 118	Hal/P 91-4

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for Halofantrine and WR 178,460 (free bases) in Blood Samples Obtained for the Protocol Titled "Efficacy of Halofantrine and Mefloquine in the Treatment of Falciparum Malaria"	1/21/92 final report	Halofantrine WR 178,460	human blood	107 107	Hal/B 91-5
Routine Analysis for Halofantrine and WR 178,460 (as free bases) of Blood Samples Obtained under the Protocol Titled "Efficacy of Halofantrine and Mefloquine in the Treatment of Falciparum Malaria"	6/23/92 final report	mefloquine	human blood	107	Mef/B 91-5
Results assoc. with Hal/P 91-1	4/28/92 final report	halofantrine WR 178,460	dog plasma	29 29	Hal/P 91-6
Routine Analysis for Mefloquine (as Free Base) in Plasma Samples Obtained under the Protocol Titled "Evaluation of the Tolerance of Prophylactic Mefloquine Regimens"	3/1/93 final report	mefloquine	human plasma	660	Mef/P 91-7
Study continued as WR6/PU 93-1	8/3/92 data	WR 6026 WR 211,789	plasma	194 194	WR6/P 92-1
Routine Analysis for Halofantrine and WR 178,460 (as Free Bases) of Plasma Samples Obtained for the Second Year of the Protocol Titled "Combined Chronic Toxicity and Oncogenicity Study of WR-171,669 HCl (Halofantrine Hydrochloride) in Rats, HWA Study No. 193-558"	3/31/93 final report	halofantrine WR 178,460	rat plasma	154 154	Hal/P 92-2
Routine Analysis for WR 238,605 (as free base) of Blood and Plasma Samples Obtained for the Protocol Titled "Rising Single Oral Dose Safety and Tolerance Study of WR 238,605 Succinate"	2/6/95 final report	WR 238,605	human plasma, blood	893 74	WR5/PB 92-3

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for WR 6026, WR 211,789 and WR 254,421 (as free bases) in Plasma and Urine Samples Obtained under the Protocol Titled "Phase II Clinical Trial of Oral WR 6026 2HCl in Patients with Vis-ceral Leishmaniasis - Initial Dose Ranging for Efficacy, Safety and Tolerance"	3/12/93 data	WR 6026 WR 6026 WR 211,789 WR 254,421	human plasma, urine	117 68 68 68	WR6/PU 93-1
Routine analysis for Halo-fantrine and WR 178,460 (as free bases) of Plasma Samples Obtained for the Protocol Titled "Pharmacokinetics of a New Multiple Dose Halofantrine Regimen"	12/10/96 in review	halofantrine WR 178,460		642 642	Hal/P 93-2
No protocol	2/25/94 data	*p*-aminohep-tanophenone	dog plasma	876	Pah/P 93-3
Routine Analysis for WR 238,605 (as free base) of Plasma Samples Obtained for the Protocol Titled "Thirteen Week Oral Toxicity Study of WR 238,605 with a Thirteen Week Recovery Period in Dogs"	4/25/94 in review	WR 238,605	dog plasma	330	WR5/P 93-4
Routine Analysis for WR 238,605 (as free base) of Plasma Samples Obtained for the Protocol Titled "Thirteen Week Oral Toxicity Study of WR 238,605 with a Thirteen Week Recovery Period in Rats"	1/20/94 final report as amended 11/4/96	WR 238,605	rat plasma	154	WR5/P 93-5
Routine Analysis for Prima-quine and Carboxyprimaquine of Serum Samples Obtained for the Protocol Titled "Primaquine and Several Recommended Prophylactic Drugs against Falciparum Malaria: Field Trial II"	5/3/96 final report	primaquine carboxy metab	human serum	60	Pri/P 93-6
Routine Analysis for Halofantrine and WR 178,460 (as free bases) of Rat Liver, Bile and Perfusate Samples	10/28/94 final data	halofantrine	rat liver perfsate bile		Hal/lpb 93-7

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for WR 238,605 (as free base) Human Plasma and Blood Samples Obtained for the Protocol Titled "Pharmacokinetics, Pharmacodynamics, Safety and Tolerance of a Single Oral Dose of WR 238605 Succinate"	9/16/94 final data	WR 238,605	human plasma blood	120 120	WR5/PB 93-8
Routine Analysis for *p*-Aminoheptanophenone of Dog Plasma Samples Obtained for the Protocol Titled "*p*-Amino-heptanophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Dogs"	2/7/95 final data	*p*-aminohep-tanophenone	dog plasma	189	Pah/P 93-9
Routine Analysis for WR 238,605 (as free base)Monkey Plasma Samples	11/22/94 final data	WR 238,605	monkey plasma	12	WR5/P 94-1
Routine Analysis for *p*-Amino-heptanophenone Rat Plasma Samples Obtained for the Protocol Titled "p-Aminohept-anophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Rats"	2/7/95 final data	*p*-aminohept-anophenone	rat plasma	152	Pah/P 94-2
Tentative Title: Routine Analysis for WR 6026 and Metabolites in Plasma and Urine Samples Obtained for the Protocol Titled "Clinical Trial of Oral WR6026•2HCl in Patients with Brazilian Visceral Leishmaniasis due to L. chagasi: Initial Dose Range Determine	1/27/97 final data more samples expected	WR 6026 WR 211789 WR 254421	human plasma urine plasma urine plasma urine plasma	 38 37 36 17 24 36 12	WR6/PU 94-3

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Tentative Title: Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "Evaluation of WR 238605 as a Prophylactic Agent against Induced P. falciparum Malaria Infection in Healthy Non-immune Subjects: A Dose Ranging Study"	11/21/94 final data	WR 238,605	human plasma blood	28 28	WR5/PB 94-4
Blind sample results to be added to SR 13B, Supplement II	10/12/94 final data	WR 238,605	dog plasma	30	WR5/P 94-5
Routine Analysis for Pyridostigmine (Cation) in Plasma Samples for the Protocol Titled "A Study to Evaluate the Safety, Tolerance, Pharmacokinetics and Pharmacodynamics of Pyridostigmine when given in Single and Multiple Doses to Males and Females in Diff	4/3/96 final report	Pyridostigmine	human plasma	2639	Pyr/P 94-6
Tentative Title: Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "A Multiple Dose Safety, Tolerance and Pharmacokinetic Study of WR 238605 when Given to Healthy Male and Female Subjects"	8/29/96 in review	WR 238605	human plasma	709	WR5/P 94-7
Tentative Title: Routine Analysis for WR 238605 in Rat Plasma Samples Obtained for the Protocol Titled "Six Month Oral Toxicity Study of WR 238605 Succinate in Rats	9/17/96 in review	WR 238605	rat plasma	405	WR5/P 95-1
Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "Evaluation of WR 238605 as a Prophylactic Agent Against Induced P. Falciparum Malaria Infection in Healthy Non-Immune Subjects II: A Multiple Dose Causal versus Suppressive	4/24/96 final data in prgrss in prgrss	WR 238605 chloroquine chiral	human plasma blood blood plasma blood	226 226 67 226 226	WR5/P 95-2

TABLE 2: PREVIOUS ROUTINE ANALYSES PERFORMED

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "WR 238605 Multiple Drug Interaction Study in Beagle Dogs"	4/26/96 samples received assay in progress	WR 238605 Mefloquine Chloroquine Quinine Doxycycline Halofantrine	human plasma	111	WR5/P 95-3
Routine Analysis for Halofantrine and WR 178460 in Plasma Samples Obtained for the Protocol Titled "Halofantrine as Prophylaxis against Malaria: Multiple-Dose Safety, Tolerance and Pharmacokinetics Study"	1/3/97 final data chiral assay in progress	Halofantrine WR 178,460 Halofantrine WR 178,460	human plasma	1060 1060 1060 1060	Hal/P 95-4
Routine Analysis for Halofantrine and WR 178460 in Aotus Monkey Blood Samples	6/4/96 samples received assay in progress	Halofantrine	monkey blood	165	Hal/B 96-1
Tentative Title: Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "Dose-Ranging Study of the Safety and Efficacy of WR 238605 in the Prevention of Relapse of Plasmodium vivax Infection in Thailand"	9/25/96 samples received assay in progress	WR238605	human plasma blood	266 266	WR5/BP 96-2
Tentative Title: Routine Analysis for Gentamicin and Paromomycin in Human Plasma Samples	12/18/96 final data more samples expected	Gentamicin Paromomycin	human plasma	36 47	Gnt/P 96-3

Purpose Of The Present Work

Work on development and/or validation of analytical methodologies during the current contract focused on assays for WR 238,605 (and its stereoisomers), halofantrine (and its metabolite and their stereoisomers), WR 6026 (and its metabolites), mefloquine (and its metabolite), artelinic acid, *p*-aminoheptanophenone (and related compounds), gentamicin and paromomycin, pyridostigmine, WR 242511, chloroquine (and its metabolites), WR 243,251,

quinine and doxycycline. Work on routine analyses of biological specimens during this period was performed for studies that required determination of concentrations of WR 238,605 (and its stereoisomers), halofantrine (and its metabolite and their stereoisomers), WR 6026 (and its metabolites), mefloquine, *p*-aminoheptanophenone (and related compounds), primaquine, gentamicin and paromomycin, chloroquine (and its metabolites), quinine, and doxycycline.

DISCUSSION

Methods Of Approach

The general development plan is described with emphasis on the laboratory procedures used. We worked on demonstrating sensitivity, specificity, linearity, lack of interferences, accuracy, and reproducibility of the analytical method, describing the extent of recovery for the method, and reporting on the stability of compounds of interest in specimens during storage and drug analysis. Validation of sensitive and specific analytical methods follow procedures described in the Analytical Section Procedural Manual, Procedure 2D-3.5, "Procedure for Validation of an Assay Methodology" and earlier versions. Methods are developed such that a single technician could complete at least 15 clinical samples in one day. These methods are to be robust and portable enough to be transported to other laboratories.

All drug standards received from the USAMRDC were logged into our record book and stored as required (protected against light, heat, or moisture). If necessary, they were checked for chemical purity or radio-purity by high pressure liquid chromatography or thin layer chromatography, purified through recrystallization or chromatography, and hydroscopic samples were dried according to USP methods.

Sample Preparation for Assay Development

Spiked samples of biological media are prepared by spiking different amounts of drug from known stock solutions into the biological media. Samples are mixed, then equilibrated for up to one hour at room temperature, unless the compound of interest is unstable, in procedures in which it is especially important for measuring drug concentrations in blood, since drugs may take some time to reach equilibrium with erythrocytes.

Sample Preparation Procedures

Suitable preparation of the biological specimens is essential for the successful application of an analytical technique. The preparation procedure should be as simple as possible, yet allow for the specific measurement of the drug in the presence of numerous biological components. The extent of sample work-up is therefore largely determined by the selectivity and sensitivity of the analytical technique. Interfering endogenous substances must be removed before analysis. A second objective in devising preparation steps for a biological specimen is to protect the analytical apparatus from contamination by proteins and undissolved

particles. Biological sample preparation thus varies according to the technical demands of the various analytical instruments utilized. Since the advent of highly selective analytical methods that combine chromatographic separation and detection in one unit [e.g. HPLC], the importance of the second objective has become more critical.

Protein Precipitation

Protein precipitation methods are rapid; they involve mixing the sample with water-miscible organic solvents. Acetonitrile yields a protein precipitate that can be readily centrifuged into a small pellet. Use of protein precipitation alone, without further work-up, is a popular application in HPLC analysis. It is possible, using appropriate measurement devices, such as electrochemical or fluorescence detectors, to obtain adequate sensitivity so that measurements in the nanogram per milliliter range can be made for drugs using small aliquots of the biological sample. We have used the protein precipitation method of sample preparation extensively in the development of analytical assays, including for antibiotics that are zwitterionic in nature, generally possess very low water-to-oil partition coefficients and, thus, are extremely difficult to extract efficiently. Also, protein precipitation is one method of choice for sample preparation, since a simpler sample preparation procedure reduces the risk of degradation. We use the direct protein precipitation method for our studies whenever possible (as demonstrated in Study Report 6 for mefloquine, Study Report 11 for WR 2721 and Study Report 12 for WR 3689).

Lower limits of quantitation with ultraviolet (UV) detectors are usually at about 50 ng/ml concentrations when the protein precipitation method is used. If UV detection is required, organic solvent extraction and solid phase extraction are more useful methods for preparation of biological samples for subsequent analysis. Extraction also limits column overloading and removes assay interferences.

Solvent Extraction

Three major variables were considered in the design of suitable organic solvent extraction procedures: the polarity of the organic solvent, the pH of the aqueous phase, and the volumes of the organic and aqueous phases (as demonstrated in Study Reports 8 and 9 for physostigmine and its metabolite eseroline in plasma and Study Report 10 for WR 6026). A higher pH is often desirable since many endogenous substances are acidic and will not be extracted at alkaline pH. Consideration of pH is therefore important even when assays are developed for neutral drugs. Lipophilic bases are quite uncommon in body fluids, so it should be relatively easy to analyze many of the lipophilic basic drugs by extracting at high pH (as shown in Study Report 13 for WR 238,605 in plasma and blood and Study Report 14 for mefloquine). However, one solvent partitioning step alone is not always capable of separating bases from acids and neutral compounds. In such cases, multiple extraction steps must be employed.

A sample preparation method combining protein precipitation with acetonitrile and extraction with organic solvent is also a viable option. This method has been successfully used in our halofantrine assay ([2] and Study Report 17).

Commercial prepacked solid phase columns [e.g. Bond Elut™] with different types of packing materials, such as silica, C2, C8, C18 and ion exchange were employed. These columns are very useful for sample purification. Two approaches can be utilized: 1] the column separates desired compound(s) from interferences, or 2] the column retains desired compound(s), undesired endogenous substances are washed away, and the desired compound(s) are eluted with a suitable solvent. For low nanogram or picogram per milliliter concentrations, the method of retaining the desired compound on the column is preferred. This method has been successfully used in our laboratory for charged, water soluble compounds (pyridostigmine (see Study Report 5 for plasma and Study Report 7 for urine)), or highly nonpolar lipophilic, weakly basic and nonvolatile compounds (WR 6026[3] and halofantrine[2]) in biological fluids. For example WR 6026 and halofantrine are non-polar lipophilic compounds which are retained on C8 columns. Pyridostigmine, a quaternary amine, will not elute with CH_3CN alone. A 2 ml CH_3CN wash after loading the biological sample onto the C8 column eliminates undesired substances. The drug is subsequently eluted with CH_3CN containing SDS and tetramethylammonium chloride (TMA+Cl-) or 1% HCl culminating in a quantitation limit of 2 ng/ml with UV detection.

Specific functional groups in molecules of interest can also be advantageously used to purify biological samples by solid phase extraction. Diol functional groups can adsorb on a boronate column and subsequently be eluted with an acidic solution. This turned out to be our method of choice in the ribavirin and WR 249,992 assay development project (see Study Report 15).

Adsorption losses to glass or other apparatus for the low level lipophilic antimalarial drugs probably explains the inconsistent results reported by many investigators. The significance of this adsorption should be considered, especially when several extraction steps are to be employed. This was demonstrated during our development of the assay for halofantrine (WR 171,669) and its active metabolite, WR 178,460, in which WR 194,965 was used as the internal standard ([2] and Study Report 17). The compounds were adsorbed by the glassware after reconstitution of the extract with organic solvent. In our experience, a true measurement of drug was obtained with the addition of a small amount of surface active agent to the solvent system before delivery onto the HPLC column. Adsorption loss can also occur in the port of delivery.

Detector Selection

The detector is a device that supplies an output in response to the presence of the compound(s) of interest. It is connected to the outlet of the column to monitor the column effluent in real time. The detector can be the most sophisticated and one of the most expensive components of a chromatographic

system. Optical detectors, which currently dominate the field for biological samples in HPLC, include UV-visible absorbance detectors and fluorescence detectors. Depending on the measured difference between incidental and transmitted light intensity, these instruments can detect down to 9 to 10 ng of sample if the direct precipitation method is used. Electrochemical (EC) detectors are also used for routine work due to their specificity and/or sensitivity.

UV-Visible Absorbance Detector

Since the analytical methods for this contract required the quantitation of nanogram per milliliter concentrations of drug in biological samples, samples assayed with the UV detector required an extensive extraction work-up. For example, the pyridostigmine plasma assay was capable of quantitating 2 ng/ml concentrations of pyridostigmine (free base) (see Study Report 5) with UV detection only because of the extensive extraction procedure.

Fluorescence Detector

Fluorescence detection is more selective than UV spectroscopy. However, more structural requirements must be met to produce a high fluorescence yield (Ø) and to allow measurement above a negligible background (i.e., better quantitation limits). Minimum detection limits for the fluorescence detector can extend below the nanogram per milliliter level for favorable samples. (See Study Reports 9, 13, and 17).

Fluorescence intensity can be manipulated both by changes in solvent components and the pH of the solvent system. For example, quinoline is non-fluorescent in hexane but fluoresces in ethanol, while indomethacin shows fluorescence at a pH above 12. Most of the synthetic antimalarial drugs are asymmetrically conjugated, not strongly ionic and, hence, would be expected to fluoresce. Fluorescence detection might therefore be expected to be the method of choice for measuring antimalarial drugs due to the sensitivity, selectivity and lower dependence on instrumental stability (from pressure and temperature changes) of the detector.

Two different light sources at various wavelengths are used in commercial fluorescence detectors. They are the deuterium and the xenon arc lamps. The xenon arc lamp has high intensity and the energy is more evenly distributed at different wavelengths, whereas the deuterium lamp emits at lower energy than the xenon arc and the intensity is drastically diminished at wavelengths above 280 nm.

Since the intensity of emitted fluorescence is dependent upon the intensity of the excitation source, it would appear that the sensitivity of a fluorescence assay can be increased without limit by using the most intense source. Many researchers do not realize that marked differences can be found with different lamp sources in commercial detectors.

8-Amino-quinoline antimalarial drugs, such as WR 6026, WR 238,605 and mefloquine (Study Reports 6, 10, 13, 14, 18, and 19) are highly conjugated and the excitation wavelengths were expected to be high. The xenon arc source equipped with monochronometers to collect both the excitation and emitted energy wavelengths provided us with maximum flexibility in fluorescence detection. With these devices, specific wavelengths for optimum sensitivity and/or selectivity were conveniently selected.

Electrochemical Detector

Electrochemical detectors (EC) are also used in methods of choice for applying liquid chromatography to trace (sub-nanogram) analysis. EC detection can provide the sensitivity and selectivity necessary for practical analytical procedures in a variety of situations. Material eluted from the chromatographic column acts at an electrode surface under controlled potential conditions and the current which results from the net exchange of electrons is monitored as a function of time. Since the amount of material converted by the electrochemical reaction is proportional to the instantaneous concentration, the current will be directly related to the amount of compound eluted as a function of time. The flow through a thin layer electrochemical cell is ideally suited for LC analysis since it can be easily constructed with a very small dead volume (1 µl) and maintain extreme sensitivity toward electroactive compounds. Several configurations using glassy carbon, carbon paste, or mercury-gold electrodes have been developed. If chromatographic conditions are carefully controlled, EC detection is quite precise and quantitative data can be obtained at the picomole level (total injected amount) for many compounds. In addition to being extremely sensitive, the electrochemical detector is quite specific in that only compounds electroactive at a given potential are detected. A large number of extremely important endogenous compounds, drugs, drug metabolites, food additives and organic pollutants are electroactive and therefore can be studied by EC. It is the method of choice for the detection of catecholamines and their analogs; numerous assay methods using EC detection have been published in the recent literature. We have been successful in using this detector for measuring the morphine analog, nalbuphine in urine. When determining whether or not a particular compound can be successfully analyzed by EC, it is not sufficient to know that the compound can react electrochemically. The type of electrode surface, the nature of the solvent system and relative ease of oxidation or reduction must be carefully considered before one can ascertain whether such an analysis is feasible (see Study Reports 11 and 12 for phosphorothioate assays). Many important compounds have been studied in detail and conditions for analysis have been optimized. In order to assess fully the possibility of developing a new assay, it is desirable to carry out voltametric studies. This is equivalent to measuring an adsorption spectrum prior to using a UV detector.

With detection in the reductive mode, analysis of blood for artesunic acid and dihydroquinghaosu had been successfully carried out in Walter Reed Army Institute of Research.[4]

Phosphorothioates ($R\text{-}SPO_3H_2$) are potential radioprotective drugs investigated by the US Army. Neither UV nor fluorescence detection is suitable for this type of compound unless some other functional group in these molecules can be derivatized. To make matters worse, phosphoro-thioates are readily hydrolyzed to free sulfhydryl compounds in vivo (metabolism) and in vitro (degradation) and possibly further oxidized to disulfides. However, phosphorothioates can be detected by EC with dual mercury/gold electrode detectors connected in series. These can be very useful for the simultaneous determination of thiols and disulfides. Two Hg/Au electrodes are utilized in a series arrangement with reduction of disulfide to thiol at the upstream electrode, followed by conventional thiol detection downstream. The upstream electrode behaves as a novel on-line post column reactor of negligible dead volume. Phosphorothioates, thiols and disulfides are all readily quantitated in this detector and suitable separation is achieved by the HPLC system. It is interesting to recall that disulfide is actually being detected as the corresponding free thiol. No confusion occurs in measurements, however, because thiols are chromatographically resolved from the disulfide and thus separately detected.

LC/MS/MS

Mass spectrometric detectors are increasingly used in methods of choice for applying liquid chromatography to trace (sub-nanogram) analysis. Our Liquid Chromatographic/Mass Spectrometric/Mass Spectrometric (LC/MS/MS) system for analysis of biological specimens requires development and validation of procedures with a PE Sciex-API III® system that uses a short liquid chromatography column (5 µm particle size, 4.6 X 50 mm), the usual liquid chromatographic mobile phases, and mass spectrometric detection with sample inlet by heated nebulizer, positive ionization by APCI (Atmospheric Pressure Chemical Ionization) and mass scanning by MRM (Multiple Reaction Monitoring) analysis.

Solvent System and Column

One of the most important steps in the development of an HPLC assay is selection of a suitable solvent system (mobile phase) and stationary phase. They are both closely related for maximum separation. Practical approaches are discussed in this section.

Reverse-Phase and Bonded Phase Columns

We intended to use reverse-phase systems for the majority of the analytical methods developed for HPLC assay described in this contract, since such bonded phase columns have several advantages for applications involving biological fluids. Reverse phase columns are stable since the stationary phase is chemically bonded to the support and cannot easily be removed or lost during use. Therefore, a pre-column and/or pre-saturation of the two phases is/are not required. Reverse-phase columns have minimal irreversible retention which is compatible with a large variety of solvents; it is often possible to inject an aqueous sample without further treatment. As a result, bonded phase columns

(BPC) are especially suited for samples containing components with widely varying K' (column capacity factor). The availability of a wide variety of functional groups on BPC packing allows reverse phase and ion paired chromatography to be carried out in a relatively simple, straight-forward manner.

In reverse-phase liquid chromatography, water is the polar solvent and any less polar, water-miscible solvent can be used in conjunction. Common examples of the second solvent include methanol, acetonitrile and tetra-hydrofuran. The design of a successful LC separation depends on matching the right mobile phase to a given column and sample ion pairing mode.

Aqueous Mobile Phase and Silica Columns

The recent use of an unbonded silica stationary phase and an aqueous mobile phase has been successfully used in our laboratory for the liquid chromatographic separation of lipophilic amines. When C18 bonded phase columns are used, it is often necessary to employ amine mobile phase modifiers to ensure good retention times and peak shapes in the ion-suppression mode. Recent work suggests that unbonded silica gel, with the maximum concentration of surface silanol groups, is a preferable stationary phase for these compounds. Use of unbonded silica as the stationary phase permits the separation of a wide variety of amine compounds with a simple mobile phase containing an organic solvent and an aqueous phosphate buffer at neutral to alkaline pH. The retention volumes are lower and the peaks are more symmetrical when silica, rather than a C18 bonded support, is used as the stationary phase. The method is especially suitable for assays of biological fluids, since endogenous non-ionic neutral lipid compounds and anionic compounds will not be retained on the silica gel column while cationic aliphatic amine drugs will be retained. The interfering substances in biological fluids are eluted at the solvent front, leaving a very clean base line about the drug's retention time. Using a silica column and an aqueous solvent system, we obtained a quantitation limit of 2 ng/ml for plasma samples for pyridostigmine (free base) (see Study Report 05).

Selectivity and Resolution Modification

As a general approach to increasing (column selectivity) and improving resolution, several options are available and can be ranked in order of decreasing promise.

Modification of Mobile Phase

Many different properties of the solvent must be considered, including solvent strength and selectivity. Polar compounds are best separated by a polar solvent system, while non-polar compounds should be separated with a less polar system. Separation may be defined as the ability of the solvent system and column material to retain the compound of interest on the column for a longer period of time than the undesired components. We found that a change from methanol to acetonitrile can sometimes enhance the selectivity of the column.

We tend to use acetonitrile as the solvent modifier since it has a lower viscosity and tends to increase the efficiency of the column; it is also characterized by increased miscibility with non-polar samples.

Change of pH and Ionic Strength

Aqueous buffers are commonly employed to suppress ionization of the ionizable sample components in reverse-phase analyses. The pH of the mobile phase is varied and the resulting changes in K' (column capacity) and alpha (column selectivity) are examined.

TABLE 3: DRUGS IN PLASMA ASSAYED WITH A SILICA GEL COLUMN AND AN AQUEOUS MOBILE PHASE

Drug	Detection Limit (ng/ml)	Detection Mode	Sample Preparation	Mobile Phase	Retention Time (min.)
WR 238605	0.8	Fluorescence	Liquid extraction	50% CH_3CN 5 mM $(NH_4)_2HPO_4$ pH = 7.0	6.50
WR 6026	1.0	UV	Liquid extraction	60% CH_3CN 5 mM $(NH_4)_2HPO_4$ pH = 7.0	7.00
Halofantrine	1.0	Fluorescence	Liquid extraction	80% CH_3OH 5 mM $(NH_4)_2HPO_4$ pH = 8.2	7.00
Pyrido-stigmine	1.4	UV	Solid Phase extraction	50% CH_3CN, 0.05% TMAC 5 mM $(NH_4)_2HPO_4$ pH = 7.2	16.4
Mefloquine	8.0	UV	Liquid extraction	80% CH_3OH 5 mM $(NH_4)_2HPO_4$ pH = 7.5	10.1

Strength of buffers or ion-pairing agents can also influence the retention times of many drugs. Most of the antimalarial drugs are highly hydrophobic in nature, hence a high ratio of organic solvent modifier should be required and ion-pair techniques will be involved.

For silica stationary and aqueous mobile phase systems, the interaction between silica and amine is electrostatic and the separation mechanism is similar to an ion-exchange mechanism. Here, the pH of the mobile phase (pH 7-9.5) and pK_a of the amine are very important in determining retention time, while the pH of the mobile phase in bonded phase systems (pH 2-5) is not as critical. Ionic strength is also critical. Thus for silica gel-aqueous mobile phase systems, mobile phase pH and ionic strength are more important to retention time determination than the organic modifier (e.g. CH_3CN, CH_3OH), which is the critical determinant in bonded gel - reverse-phase systems.

Change of Stationary Phase

A change of stationary phase is less convenient than a change in mobile phase composition and is less commonly used. Further adjustment of the mobile phase composition is usually required when a new column packing or stationary phase is used to optimize of solvent strength and K' values.

Most aromatic antimalarial drugs are very non-polar. It is reasonable to expect that the retention time will be shorter on a more polar C8 column (tend not to retain) than a non-polar C18 column. This was found to be true during the development of our first WR 171,669 (halofantrine) assay. The retention time for this compound is reduced by 1/3 by changing from a C18 column to a C8 column when the same mobile phase is used.

Temperature Change

The fourth technique for varying K' values is to increase (or decrease) the temperature. Since an increase in temperature normally reduces all sample K' values, it is usually necessary to decrease solvent strength to compensate for this effect. A change in temperature usually has little effect on sample K' values in liquid-liquid chromatography, but it is important in ion exchange and ion-pair chromatography. For this reason, a change in temperature for improvement of K' in ion-exchange and ion-pair chromatography is generally more promising than a change in stationary phase.

Complexation

A final means of changing K' values, sometimes dramatically, is through chemical complexation. A well known example is the use of metal ions (e.g. $Ag^+NO_3^-$) in the solvent system to separate various olefinic compounds. The complexation of olefin and metal ion causes dramatic changes in retention time and selectivity.[5] This technique is probably applicable for some antimalarial drugs.

For the most part, we intended to use C8 columns and/or ion-pair techniques to develop assay methodologies. However, silica gel column - aqueous mobile phase systems are our general method of choice for amines. Since measurement concentrations of 5 to 20 ng/ml are required, we expected to use 5 µm particle size columns for separation of drugs.

Derivatization

Derivatization is an important adjunct to HPLC assays. The choice of derivatization procedure is dependent upon the type of detector that is used. We are actively involved in pre-column and post-column derivatization as well as in structure modification studies to increase detection sensitivity. A wealth of information on potentially useful derivatives is available from the disciplines of qualitative organic analysis[6] and protective group synthesis.[7] In choosing a derivative for HPLC, ideally the reaction should be specific, quantitative, free from side reactions, complete in a relatively short time, and done under mild conditions. This kind of information is not readily available in the literature, and therefore, derivatization studies can be a rather time consuming venture. The design or choice of a derivatizing agent is critical.

Post column derivatization or degradation is also an excellent way to increase sensitivity of an assay. The technique of post column hydrolysis at alkaline pH and post column oxidation reactions with potassium permanganate or potassium periodate can be applied to assays that employ fluorescence detection. Post-column photo-irradiation is another way to increase sensitivity. First, the drug of interest is separated from other components of the sample by HPLC. Then, the sensitivity is enhanced by photo-irradiation which may rearrange the chromophore or otherwise break bonds to form a fluorescent species

Assay Validation

Validation of the methods were performed using biological fluids obtained from same species, when possible. This process indicates sample stability, method precision, accuracy and selectivity, and the feasible sample concentration range for use in pharmacokinetic or bioavailability studies. Validation procedures are part of our standard operating procedures (SOP) which are written in accordance with our program to meet Good Laboratory Practice (GLP) regulations. The procedures are described in the Analytical Section Procedural Manual, Procedure 2D-3.2 "Procedure for Validation of an Assay Methodology." This section summarizes Procedure 2D-3.2.

Specificity

The specificity should be evidenced by showing with chromatograms that: Test compounds are separated from major metabolites (if metabolite standard is available); Test compounds are separated from co-administered drugs (if any); At least three different sources of biological fluid should be free of possible interference by endogenous peaks at the retention times of the test compounds.

All assay methods developed required use of an internal standard. Analogs of the compounds under study or chemicals with similar functional groups were preferred as internal standards. The internal standard must elute at a different time than the drug of interest, yet separate from endogenous substances in the biological sample. In addition, it should have similar extraction or partition properties as the drug of interest during the sample preparation process.

Linearity

Linearity is demonstrated by acceptable spiked vs. calculated concentrations (or vs. peak response ratios), y-intercept, and coefficient of determination (r^2) values for the standard curve.

Calibration curves were constructed from the peak height (or peak area) ratio of drug to internal standard versus spiked concentration of drug by linear regression (unweighted or weighted method).

In the weighted least squares linear regression method, weights (w) = $1/y_i$, the intercept, b, is defined by:

$$b = \frac{\left(\left(\sum w_i x_i^2\right)\left(\sum w_i y_i\right) - \left(\sum w_i x_i\right)\left(\sum w_i x_i y_i\right)\right)}{\left(\left(\sum w_i\right)\left(\sum w_i x_i^2\right) - \left(\sum w_i x_i\right)^2\right)}$$

and the slope, m, is defined by:

$$m = \frac{\left(\left(\sum w_i\right)\left(\sum w_i x_i y_i\right) - \left(\sum w_i x_i\right)\left(\sum w_i y_i\right)\right)}{\left(\left(\sum w_i\right)\left(\sum w_i x_i^2\right) - \left(\sum w_i x_i\right)^2\right)}$$

Two standard curves may be calculated from the same set of standard curve calibrators (unless the weighted linear regression method is used). The low range curve is calculated from low concentration standard curve points and is used to derive concentrations from samples with peak response ratios at or below the calculated peak response ratio of the highest standard curve point used in the low range curve. The high range curve is calculated from all standard curve points and is used to derive concentrations from samples with peak response ratios above the calculated peak response ratio of the highest standard curve point used in the low range curve.

Standard curve results are reported in a table containing spiked concentrations, peak response ratios, calculated concentrations, slope(s), intercept(s) and r^2 value(s) of a typical standard curve that was used in the method validation and in a table containing all slopes, intercepts and r^2 values of standard curves run in "intraday" and "interday" studies.

Lower Limit of Quantitation

The lower limit of quantitation is defined as the lowest standard curve concentration which can be reasonably, accurately, and precisely quantitated. Six samples spiked to the lowest standard curve concentration and a standard curve are prepared. The samples are run together within one day (or one run). The 6 lowest point of the standard curve sample concentrations, and their mean, S.D., C.V. (percent) and deviation (percent) are calculated. These data are used as the quantitation limit intraday result.

The 6 calculated lowest point of the standard curve concentrations that were obtained in the interday precision study and their means, S.D.s, C.V. percents and deviation percents are used as the quantitation limit interday result.

Recovery

It is important to check the recovery of compounds of interest during the assay in order to assess the uniformity of recovery during the assay or whether or not a better recovery can be obtained. Radio-labeled drugs, when necessary, were added to the sample and either the direct precipitation, solid phase purification or the extraction procedure was utilized to evaluate recovery. If labeled compounds were not available, a recovery study similar to those for WR 6026,[3] halofantrine and its metabolite, WR 178,460,[2] and pyridostigmine[8] were carried out. In brief, the recoveries of these drugs from plasma or whole blood were determined by comparison of the drug-to-internal standard peak height ratios of blood or plasma versus water samples spiked with the drug. In each case, the internal standard was added after sample was eluted from the solid phase column, extraction from organic solvent, or direct precipitation with CH_3CN to insure that the internal standard did not bind to the blood or plasma or to the cartridge during the preparation.

Precision

Precision is expressed as the standard deviation (S) of the assayed concentration where Xi are the repeated concentration measurements of an individual sample and $\overline{x}$ is the mean concentration.

$$S = \left(\frac{\sum_{i=1}^{N} (Xi - \overline{x})2}{(N - 1)} \right)^{\frac{1}{2}}$$

The coefficient of variation (C.V.) was used for determination of the precision. The sample number was 6 for intraday and 12 for interday precision. The bias of an assay method is determined by comparing biological sample results with spiked values. The significance of the bias is established by setting a confidence limit.

$$\text{Percent C.V.} = \frac{S \bullet 100}{\overline{x}}, \quad (N \geq 6)$$

If needed, the assay results were compared to those obtained with an assay of proven reliability and specificity. For example, the Pearson correlation coefficient (r) can be used. Maximum r value indicates exact correlation between the two variables and r = 0 indicates complete independence.

$$r = \frac{(Si - \overline{s})(Yi - \overline{y})}{n \bullet Sx \bullet Sy}$$

The within-run precision was determined by measuring the amount of drug in a number of biological samples, in duplicate. The duplicate mean results are used to calculate the standard deviation. The between-run precision is measured on separate days with replicate samples at low, intermediate and high drug concentrations. From these three sets of replicate samples, the between-run standard deviation is calculated for each drug concentration.

Accuracy

Accuracy was determined by assaying a series of blind samples prepared according to the project director of DAMD. Estimates of the accuracy of the method over the standard curve working range were also determined in the precision analysis by the analysis of replicate spiked samples for intraday (n = 6) and interday (n = 12) precision. Results were expressed as relative error (RE) with respect to the spiked concentration.

Stability

Stability studies of a drug in biological media serve to establish the procedure for proper storage of the samples and furnishes information to clinical researchers on how best to handle these occasionally labile samples. We have a great deal of experience in planning and executing the required stability studies. In methods developed for analytical and clinical studies, drug stability may play a particularly important role.

Known amounts of sample in different biological media are measured at various times after preparation and assayed for the drug, in duplicate. Variables, including light exposure, storage conditions (container type) and pH of the biological samples, are evaluated if necessary.

Since clinical samples are often repeatedly assayed and samples are thawed and refrozen, it is necessary to check for any instability of samples during these processes. Practically, this study can be done by using two concentration samples (High and Low). The same volume of biological fluid used to prepare standard curve samples is aliquoted to the appropriate number of tubes. Samples (in duplicate) are thawed and refrozen (a cycle) for 5 cycles. Samples are repeatedly thawed and refrozen according to the following table. Samples are thawed as if for sample preparation to room temperature and are left to stand at room temperature for 1 hour.

Cycle	Keep these samples in freezer
1	a
2	a, b
3	a, b, c
4	a, b, c, d
5	a, b, c, d, e

Following Cycle 5, all of the samples are thawed to room temperature and assayed with a standard curve. Test sample concentrations are calculated and reported in a table for each concentration (n=2) of mean concentrations (n=2) at each test point (n=5).

System (processed sample) stability: Stability of drug and internal standard in biological samples prepared for analysis as described above under "Sample Preparation" were demonstrated by assay of sets of control samples with concentrations to cover the standard curve range. Assay results were obtained for prepared samples that were left standing at room temperature for various times after preparation.

Refrigerated (processed sample, refrigerated) stability: Assay results were obtained for prepared samples that were left standing at 4°C for various times after preparation.

Long term stability: Stability of a drug in a biological specimen stored at -20°C and/or -70°C was demonstrated by assay of sets of control samples prepared as described under "Sample Preparation" at concentrations to cover the standard curve range. Long term stability samples were kept at -70°C or -20°C until prepared and analyzed.

Bench top (unprocessed sample) stability: Plasma samples were left to stand at room temperature for various times after generation, then were kept at -70°C until prepared and analyzed.

Purity of Standard Chemicals

The standard chemicals used in a study are USP™ reference standards (if available) or pure chemicals provided by the sponsor, unless otherwise instructed.

A chemical purchased from a general chemical company is not used as a standard, except in unusual circumstances and when its purity can be verified against a USP™ reference standard or the sponsor's standard verified by a certificate of analysis. The internal standard is not under this restriction.

USP™ reference standards can be regarded as 100% pure (unless specified), and no purity correction factor for concentration calculations is necessary. However, a sponsor's standard chemical must be regarded as possibly impure and a correction factor should be considered.

When verifying non USP™ reference standard chemical purity, the working standard solution is run under the method used for assaying biological samples. Each solution is injected 3 times, and two standard solutions are prepared for each standard chemical.

A copy of the supplier's certification sheet is saved with the method validation files.

Routine Assay Procedure

The following are the steps carried out when samples arrive for routine analysis. Sample arrival is recorded in the sample log-in book and on the log-in sheet, which includes the name of the shipper, arrival date, number of samples, sample storage location, and sample condition. An analytical procedure (AP) is normally completed prior to routine analysis. In this AP, the method description is condensed to about 3-10 pages that contain information regarding instrumentation, assay conditions, source of chemicals, preparation of stock solutions, sample preparation and representative chromatograms.

For routine sample analysis, standard curve, blank and control samples are also analyzed. Sets of equipment consisting of a pump, detector, column, integrator and autosampler or the LC/MS/MS system were set up for routine assay. Each system is tested by assigning personnel to run a series of controls; the performance of equipment and technical personnel are validated before routine sample assay.

Carry over testing is performed for each run by assay of a blank sample (not spiked with internal standard or with drug standard) immediately following the assay of a high concentration control.

To monitor variation during the course of assay of a sufficient quantity of samples, a series of controls are prepared beforehand and stored in the freezer. Control samples are run together with standards and routine assay samples. Each set of controls normally includes three different concentrations within the range of the standard curve. For every group, treatment, or up to 20 routine assay samples, a set of controls (e.g. low, medium and high) is included before and after the set to validate the results. Since the concentrations of the controls are known, it is possible to judge whether the routine assay samples must be repeated on the basis of the results obtained for the controls.

Assay samples are prepared by spiking known volumes of biological sample with a known amount (constant over all samples) of internal standard (IS). Standard curve samples are generated by spiking interference free biological samples with known amounts of standard compound and IS. These standard curve and assay samples are prepared according to the analytical procedure, then injected onto an LC column for separation and subsequent detection. The peak response ratio of standard compound to IS is calculated for each sample from the measured peak response obtained by HPLC or LC/MS/MS. Finally, spiked concentrations and standard compound to IS peak response ratios of the standard curve samples are fit by weighted or non weighted least squares linear regression to the equation for the best straight line ($y = mx + b$, where y = peak response ratio and x = standard compound concentration), and standard compound concentrations in assay samples are calculated by this equation from the standard compound to IS peak response ratios obtained by HPLC or LC/MS/MS.

Assay findings are then sent in a report with a complete assay methodology including detailed methods, statistical evaluation of methods, routine assay sample results, results from the control samples, and one representative set of calibration chromatograms. Results can be sent by disc or through a modem for pharmacokinetic evaluation.

Experimental methods

The goals of the research under contract DAMD17-97-C-7058 are 1) to develop and validate methods to assay for drug substances in biological fluids for pharmacokinetic, bioavailability, drug metabolism and drug monitoring studies, and 2) to use these methods to perform routine analyses of biological specimens to support pharmacokinetic and bioavailability studies as part of preclinical and clinical investigations undertaken for the purpose of new drug development.

Method Development and/or Validation Results

The following section describes the status of specific methods developed and validated or currently being developed and/or validated during the contract.

TABLE 4: CURRENT STUDY REPORTS

Report No.	Report Date	Report Title	Test Article	Test System	Lower Limit of Quantitation
13	5/16/96 (accepted as final 3/3/98)	Supplement II: Quantitation of WR 238605 as Free Base in Dog Plasma by HPLC and Fluorescence Detection	WR 238,605	Dog Plasma	1.00 ng/ml
17B	1/23/98 final report	Supplement I: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Perfusate by Precipitation and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat perfusate	0.520 µg/ml 0.510 µg/ml
17B	1/28/98 final report	Supplement II: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Perfusate by Extraction and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat perfusate	10.4 ng/ml 10.2 ng/ml
17B	1/28/98 final report	Supplement III: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Bile by Precipitation and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat bile	0.416 µg/ml 0.408 µg/ml
17B	1/28/98 final report	Supplement IV: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Bile by Extraction and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat bile	20.4 ng/ml
17B	1/28/98 final report	Supplement V: Quantitation of Halofantrine and WR 178,460 (as Free Bases) in Rat Liver by Precipitation and HPLC with a Silica Gel Column and an Aqueous Mobile Phase	Halofantrine WR178460	rat liver	0.540 µg/ml 0.540 µg/ml
18	Final report in preparation	Quantitation of WR 6026 and WR 211,789 (WR 6026 Metabolite) in Plasma and Blood by HPLC with a Silica Gel Column and an Aqueous Mobile Phase	WR 6026 WR 211789	Plasma Blood	0.980 ng/ml 1.21 ng/ml
19	1/14/92 in revision	Quantitation of Mefloquine in Human Blood By HPLC, Extraction Method	mefloquine WR 160972	human blood	7.36 ng/ml

TABLE 4: CURRENT STUDY REPORTS

Report No.	Report Date	Report Title	Test Article	Test System	Lower Limit of Quantitation
21	Draft report in preparation	Tentative title: Quantitation of *p*-Aminoheptanophenone, *p*-Aminooctanophenone, and *p*-Aminopropiophenone in Dog Plasma by HPLC	PAHP PAPP PAOP	dog plasma	4.08 ng/ml 4.04 ng/ml 4.16 ng/ml
22	Draft 7/18/94 in revision	Quantitation of WR 6026, WR 211,789, and WR 254,421 (as Free Bases) in Human Urine By HPLC	WR 6026 WR 211,789 WR 254,421	human urine	5.17 ng/ml 5.09 ng/ml 45.4 ng/ml
24	10/21/97 final report	Quantitation of Paromomycin and Gentamicin in Human and Rat Plasma by HPLC	Gentamicin Paromomycin Gentamicin Paromomycin	human plasma rat plasma	0.1 µg/ml 0.1 µg/ml 0.1 µg/ml 0.1 µg/ml
26	12/12/96 final report, amendment in preparation	Quantitation of WR 242511 (as Free Base) in Human and Dog Plasma By HPLC with a Silica Gel Column and an Aqueous Mobile Phase	WR 242511 WR 242511	human plasma dog plasma	4.00 ng/ml 4.00 ng/ml
27	12/17/97 final report	Quantitation of WR 238605 *R&S* Enantiomers (as Free Bases) in Human Plasma by HPLC	*R* WR 238605 *S* WR 238605	human plasma	5 ng/ml 5 ng/ml
28	Draft in preparation	Tentative title: Quantitation of R&S Isomers of Halofantrine and WR 178,460 in Human Plasma by HPLC	Halofantrine WR 178,460	human plasma	
29	8/22/97 status report	Validation of a LC/MS/MS Method for the Determination of Chloroquine and Monodesethyl-chloroquine in Human Blood Samples	Chloroquine Desethyl-chloroquine	human plasma	20 ng/ml 20 ng/ml
30	In validation	Tentative title: Quantitation of WR 243251 in Human Plasma by LC/MS/MS	WR 243251	human plasma	1 to 5 ng/ml

TABLE 4: CURRENT STUDY REPORTS

Report No.	Report Date	Report Title	Test Article	Test System	Lower Limit of Quantitation
31	Draft in preparation	Tentative title: Quantitation of WR 238,605, Mefloquine, Chloroquine, Quinine and Doxycycline in Dog Plasma by LC/MS/MS	WR 238605 Mefloquine Chloroquine Quinine Doxycycline	dog plasma	
32	Draft in preparation	Tentative title: Quantitation of WR 238,605 in Human Plasma and Small Volume in Human Blood by LC/MS/MS and	WR 238605	human plasma blood	
33	In validation	Tentative title: Quantitation of Halofantrine and WR 178,460 in Human Plasma by LC/MS/MS	Halofantrine WR 178,460	human plasma	
34	In development	Tentative title: Quantitation of WR 254421 in Human Plasma by LC/MS/MS	WR 254421	Human plasma	
35	In development	Tentative title: Quantitation of Artelinic Acid in Rat Plasma by LC/MS/MS	Artelinic Acid	Rat plasma	

Study Report 13B: Quantitation of WR 238,605 in Human Plasma and Blood and Rat and Dog Plasma

Study Characteristics: Study Report 13B	
Test Article:	WR 238,605
Test System:	human plasma and blood
Internal Standard:	WR 6026
Sample Assay Volume:	0.2 ml
Sample Cleanup:	extract with methyl *t*-butyl ether
Analytical System	
Detector:	Fluorescence with excitation - 375 nm; emission - 480 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5μ particle size
Mobile Phase:	acetonitrile/water (1:1, v/v) final concentration of 5 mM $(NH_4)_2HPO_4$ at pH 7.0.
Validation Results: human plasma	
Quantitation Limit:	0.815 ng/ml
Standard curve range:	0.815-408 ng/ml
Interday Precision	
Concentration Range:	1.63-163 ng/ml
CV Range:	6.64-8.70%
Intraday Precision	
Concentration Range:	1.63-163 ng/ml
CV Range:	5.44-9.07%
Blind Sample Assay	
Concentration Range:	1.20-179.4 ng/ml
Bias Range:	-4.68 to +31.7%
Mean Recovery:	82.5%
Stable Plasma Storage:	-20°C for 4 months
Validation Results (continued): human blood	
Quantitation Limit:	1.91 ng/ml
Standard curve range:	1.91-383 ng/ml
Interday Precision	
Concentration Range:	3.82-143 ng/ml
CV Range:	2.94-7.75%
Intraday Precision	
Concentration Range:	3.82-143 ng/ml
CV Range:	7.69-9.15%
Blind Sample Assay	
Concentration Range:	2.39-239.16 ng/ml
Bias Range:	-22.7 to -5.86%
Mean Recovery:	97.3%
Stable Blood Storage:	-20°C for 30 days

Study Description: WR 238,605 in Human Plasma and Blood

WR 238,605 succinate (N4-[2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl) phenoxy]-8-quinolinyl]-1,4, pentanediamine succinate), an 8-aminoquinoline derivative, has been chosen by the U.S. Army Drug Development Program to be developed as an anti-malarial drug to replace primaquine.[9] Although primaquine is the best available drug for the curative treatment of *Plasmodium vivax* malaria[10], problems associated with its toxicity[11] and the development of resistant strains,[12] led to the development of WR 238,605 succinate, which was found to be 12.8 times more potent on a molar basis than primaquine in curing the rhesus monkey of *P. cynomolgi*, the simian equivalent of *P. vivax* .[9]

Plasma samples were analyzed for WR 238,605 (free base) (N4-[2,6-dimethoxy-4-methyl-5-[(3-trifluoromethyl)phenoxy]-8-quinolinyl]-1,4, pentanediamine base), an 8-aminoquinoline derivative, with an HPLC procedure that uses a silica gel column, an (acetonitrile/water) aqueous mobile phase, a fluorescence detector, and 0.2 ml plasma samples. Sample cleanup consisted of extraction into methyl *t*-butyl ether, evaporation of the organic phase and reconstitution of plasma sample extracts in acetonitrile/ water prior to separation by HPLC. The method SOPs contains detailed procedures and results, which are summarized below.

Assay samples were prepared by spiking known plasma volumes with a known amount (constant over all samples) of WR 6026 internal standard (IS). Standard curve samples were generated by spiking interference free plasma samples with known amounts of WR 238,605 (free base) and IS. Standard curve and assay samples were extracted, then injected onto an HPLC column for separation and subsequent fluorometric detection. The peak height ratio of WR 238,605 (free base) to IS was calculated for each sample from the measured peak heights obtained by HPLC. Finally, spiked concentrations and WR 238,605 (free base) to IS peak height ratios of the standard curve samples were fit by least squares linear regression to the equation for the best straight line ($y = mx + b$, where y = peak height ratio and x = WR 238,605 (free base) concentration), and drug concentrations in assay samples were calculated by this equation from the WR 238,605 (free base) to IS peak height ratios obtained by HPLC.

Plasma samples (0.2 ml aliquots unless otherwise specified) to be assayed for WR 238,605 (free base) were pipetted into glass culture tubes. Next, 20 µl of internal standard (WR 6026, 6-methoxy-8-(6-diethylamino-hexylamino lepidine dihydrochloride) solution (27.9 µg/ml) and 0.1 ml of 0.1 N NaOH buffer were added and vortexed. Then, 3 ml of methyl *t*-butyl ether extracting solvent was added, and the samples were vortexed for 1 min, twice, and centrifuged for 10 min at 3000 *g*. The organic layer of each sample was pipetted to a clean tube and evaporated to dryness under nitrogen. The residue was reconstituted with 200 µl of acetonitrile/water (1:1, v/v), transferred to a WISP insert, and injected onto the HPLC column.

Study Characteristics: Short Validation Supplement I to SR 13

Test System:	Rat Plasma
Validation Results:	
Quantitation Limit:	1.00 ng/ml
Precision CV:	6.72%
Precision Error:	21.8%
Standard curve range:	1.00-400 ng/ml
Interday Precision	
Concentration Range:	2.04-204 ng/ml
CV Range:	0.54-6.46%
Intraday Precision	
Concentration Range:	2.04-204 ng/ml
CV Range:	4.62-10.1%
Mean Recovery:	66.5%

Study Description: WR 238,605 in Rat Plasma (the methodology was presented in DAMD17-92-C-2028 final report)

The original validation Study Report (No. 13B) for WR 238,605 described validation for human plasma and blood. To satisfy requirements of our current laboratory SOP on validation, a short validation study was performed, Study Report 13B, Supplement I titled "Quantitation of WR 238,605 as Free Base in Rat Plasma by High-Performance Liquid Chromatography and Fluorescence Detection," consisting of precision (a shortened 3 run interday analysis and the normal intraday analysis) and recovery tests with rat plasma.

Study Characteristics: Short Validation Supplement II to SR 13

Test System:	Dog Plasma
Validation Results:	
Quantitation Limit:	1.00 ng/ml
Interday CV:	15.0%
Interday Error:	25.3%
Standard curve range:	1.00-400 ng/ml
Interday Precision	
Concentration Range:	2.04-204 ng/ml
CV Range:	4.60-10.2%
Intraday Precision	
Concentration Range:	2.04-204 ng/ml
CV Range:	1.20-10.8%
Mean Recovery:	66.4%

Study Description: WR 238,605 in Dog Plasma (the methodology was presented in DAMD17-92-C-2028 final report)

An additional short validation study, Study Report 13B, Supplement II titled "Quantitation of WR 238,605 as Free Base in Dog Plasma by High-Performance Liquid Chromatography and Fluorescence Detection," with dog plasma was performed.

Study Report 17: Halofantrine and Metabolite in Human Plasma and Blood, Rat Liver, Rat Bile and Rat Perfusate

Study Characteristics: Study Report 17	
Test Article:	halofantrine, WR 178,460
Test System:	human plasma and blood
Internal Standard:	procainamide hydrochloride
Sample Assay Volume	0.5 ml
Sample Cleanup:	Precipitate with acetonitrile extract with methyl *t*-butyl ether
Analytical System	
Detector:	fluorescence
Column Type:	silica
Column Size:	4.6x250 mm, 5μ particle size
Mobile Phase:	CH_3OH/water (80:20, v/v) 5 mM $(NH_4)_2HPO_4$
Validation Results: halofantrine in human plasma	
Quantitation Limit:	0.960 ng/ml
Standard curve range:	0.960-115 ng/ml
Interday Precision	
Concentration Range:	1.92-76.8 ng/ml
CV Range:	4.24-10.6%
Intraday Precision	
Concentration Range:	1.92-76.8 ng/ml
CV Range:	3.82-13.6%
Mean Recovery:	73.0%
Stable Plasma Storage:	-80°C for 4 months
Validation Results: WR 178,460 in human plasma	
Quantitation Limit:	0.928 ng/ml
Standard curve range:	0.928-111 ng/ml
Interday Precision	
Concentration Range:	1.86-74.2 ng/ml
CV Range:	4.90-6.65%
Intraday Precision	
Concentration Range:	1.86-74.2 ng/ml
CV Range:	4.21-7.94%
Mean Recovery:	95.5%
Stable Plasma Storage:	-80°C for 4 months

Validation Results: halofantrine in human blood	
Quantitation Limit:	0.960 ng/ml
Standard curve range:	0.960-115 ng/ml
Interday Precision	
Concentration Range:	1.92-76.8 ng/ml
CV Range:	3.42-10.5%
Intraday Precision	
Concentration Range:	1.88-75.4 ng/ml
CV Range:	4.60-10.0%
Mean Recovery:	68.6%
Stable Blood Storage:	-80°C for 4 months
Validation Results: WR 178,460 in human blood	
Quantitation Limit:	0.928 ng/ml
Standard curve range:	0.928-111 ng/ml
Interday Precision	
Concentration Range:	1.86-74.2 ng/ml
CV Range:	6.49-7.88%
Intraday Precision	
Concentration Range:	1.85-73.9 ng/ml
CV Range:	5.70-9.40%
Mean Recovery:	95.9%
Stable Blood Storage:	-80°C for 4 months

Study Description: Halofantrine and Metabolite in Human Plasma and Blood (the methodology was presented in DAMD17-92-C-2028 mid-term report)

Study Report 17 presents a second approach to the HPLC analysis of blood and plasma samples for determination of the free base concentrations of halofantrine (WR 171,669) and of its metabolite (WR 178,460). Study Report 4 (dated August 23, 1985 under contract DAMD 17-83-C-3004) describes an ion-paired liquid chromatographic assay for halofantrine and its metabolite as free bases in blood & plasma. The assay involves protein precipitation and a column elution step prior to HPLC separation. However, due to successes in this laboratory in assays for several amines in which a silica gel stationary phase was used[13,14] the same approach was tried in an assay for halofantrine and its metabolite (as free bases). The second method involves the use of a silica gel column run with an aqueous mobile phase which results in cleaner baselines and a higher signal to noise ratio at concentrations similar to the ion-paired method.[15] All glassware used in this assay must be silanized to limit error due to absorption.

Plasma samples for analysis are pipetted (0.5 ml) into silanized tubes. Approximately 1.5 ng of the internal standard, procainamide hydrochloride, and 1 ml of CH_3CN to precipitate the proteins were added. The samples are vortexed and centrifuged and the resulting supernatant is transferred to a

silanized tube and evaporated under N_2 to about 0.5 ml. Water (0.5 ml) and 0.1 N NaOH buffer (0.5 ml) were added to make the solution alkaline. Methyl *t*-butyl ether (5 ml), the extracting solvent, is added and the sample is vortexed, centrifuged and frozen in a dry ice/MeOH bath. The organic layer is poured into a 13x100 mm silanized tube. This extraction step is repeated with another 5 ml of extracting solvent which is poured into the same 13x100 mm silanized tube. The solvent is evaporated to dryness under N_2, the residue is reconstituted with 200 µl of methanol/water (80:20) containing 0.001% HCl, and 25 to 150 µl is injected onto the column.

Blood samples are treated similarly except 0.5 ml water to lyse the cells and 2 ml of CH_3CN to precipitate the proteins were added. For standard curve samples, appropriate amounts of drug and metabolite are added, and samples are left to equilibrate at room temperature for one hour.

A silica gel column and a mobile phase composition of methanol/water (80:20, v/v) with a final concentration 5 mM $(NH_4)_2HPO_4$ were used to separate halofantrine and WR 178,460 (as free bases) from the internal standard and interfering endogenous substances in an isocratic elution. In typical chromatograms, halofantrine and WR 178,460 (as free bases) for both plasma and blood are sufficiently separated from each other and from endogenous compounds to permit successful sample assay. Halofantrine (free base) eluted at 8 minutes, WR 178,460 (free base) eluted at 11 minutes, and the internal standard eluted at 26 minutes.

A linear relationship was demonstrated between the halofantrine and WR 178,460 (as free bases) concentrations in plasma or blood and the peak height ratios of the halofantrine or WR 178,460 peak to the internal standard peak. The minimum detection limits, 0.928 and 0.960 ng/ml, were determined as the halofantrine and WR 178,460 (as free bases) concentrations, respectively, at which the signal to noise ratio was 3 to 1. Due to the extent of the standard curve range and in order to obtain more accurate determinations of low level drug or metabolite (as free bases) concentrations, two standard curves were constructed from the same set of standard curve data points.

Data points from 0 to 14.4 ng/ml were used to construct a low halofantrine (free base) concentration standard curve with which to calculate the low concentration samples. All data points (0 to 115 ng/ml) were used to construct a high halofantrine (free base) concentration standard curve for the high concentration samples. Concentrations of samples with peak height ratios above that calculated at 14.4 ng/ml from the low concentration standard curve were calculated with the high concentration standard curve.

Data points from 0 to 13.9 ng/ml were used to construct a low WR 178,460 (free base) concentration standard curve with which to calculate the low concentration samples. All data points (0 to 111 ng/ml) were used to construct a high WR 178,460 (free base) concentration standard curve for the high concentration samples. Concentrations of samples with peak height ratios above

that calculated at 13.9 ng/ml from the low concentration standard curve were calculated with the high concentration standard curve.

For representative standard curves, the coefficients of determination for halofantrine (free base) in plasma were 0.9995 and 0.9950 and for halofantrine (free base) in blood were 0.9985 and 0.9985 for low and high concentration ranges, respectively. The coefficients for WR 178,460 (free base) in plasma were 0.9998 and 0.9983 and for WR 178,460 (free base) in blood were 0.9956 and 0.9993 for low and high concentration ranges, respectively.

Blind blood samples, prepared April 1, 1993 were assayed by the method described in Study Report 17, modified by incorporation of an analog to halofantrine (WR 122,455) as an alternate internal standard. The results, and changes induced by using a different internal standard, will be incorporated in Study Report 17B.

Study Characteristics: Study Report 17B

Test Article:	halofantrine, WR 178,460
Test System:	human plasma and blood
Internal Standard:	WR 122,455
Sample Assay Volume:	0.5 ml
Sample Cleanup:	Precipitate with acetonitrile Double extraction with methyl *t*-butyl ether

Analytical System

Detector:	fluorescence-Ex: 300 nm, Em: 375 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5µ particle size
Mobile Phase:	CH_3OH/water (80:20, v/v) 5 mM $(NH_4)_2HPO_4$

Validation Results: halofantrine in human plasma

Lower Limit of Quantitation:	2.08 ng/ml
Interday Mean, CV and RE:	1.81 ng/ml, 12.0% and -13.0%
Intraday Mean, CV and RE:	1.97 ng/ml, 18.2% and -5.67%
Standard curve range:	2.08 to 266 ng/ml
Interday Precision Concentrations:	4.04, 10.1, 40.4, and 129 ng/ml
CV Range:	7.17 to 14.2%
RE Range:	-1.87 to +3.31%
Intraday Precision Concentrations:	2.04, 10.2, 61.2, and 102 ng/ml
CV Range:	3.83 to 8.75%
RE Range:	-16.3 to -1.00%
Blind Sample Assay	Run in conjunction with Study Report 17
Overall Mean Recovery:	46.4%
Stability	
Plasma Freezer Storage:	-80°C for 4 months
Processed Sample:	Room temp. for 1 day
Plasma Storage:	On ice for 4 hours
5 Cycle Freeze/Thaw:	5 cycles to -70°C
Standard Solution:	6 months

Validation Results: WR 178,460 in human plasma

Lower Limit of Quantitation:	2.08 ng/ml
Interday Mean, CV and RE:	2.02 ng/ml, 13.3% and -3.06%
Intraday Mean, CV and RE:	2.11 ng/ml, 4.07% and +1.52%
Standard curve range:	2.08 to 266 ng/ml
Interday Precision Concentrations:	4.16, 10.4, 41.6, and 133 ng/ml
CV Range:	4.19 to 12.3%
RE Range:	-0.149 to +3.91%
Intraday Precision Concentrations:	1.93, 9.64, 57.9, and 96.5 ng/ml
CV Range:	1.62 to 6.95%
RE Range:	-3.49 to +6.11%
Blind Sample Assay	Run in conjunction with Study Report 17
Overall Mean Recovery:	80.9%
Stability	
Plasma Freezer Storage:	-80°C for 4 months
Processed Sample:	Room temp. for 1 day
Plasma Storage:	On ice for 4 hours
5 Cycle Freeze/Thaw:	5 cycles to -70°C
Standard Solution:	6 months

Validation Results: halofantrine in human blood

Lower Limit of Quantitation:	1.02 ng/ml
Interday Mean, CV and RE:	1.14 ng/ml, 12.2% and +11.4%
Intraday Mean, CV and RE:	1.12 ng/ml, 10.1% and +9.45%
Standard curve range:	1.02-245 ng/ml
Interday Precision Concentrations:	4.16, 10.4, 41.6, and 133 ng/ml
CV Range:	5.38 to 10.8%
RE Range:	-3.12 to +5.08%
Intraday Precision Concentrations:	2.04, 10.2, 61.2, and 102 ng/ml
CV Range:	9.58 to 16.7%
RE Range:	-9.31 to +0%
Blind Sample Assay	
Concentration Range:	2.04 to 183.6 ng/ml
RE Range:	-8.21 to +3.68%
Overall Mean Recovery:	80.8%
Stability	
Plasma Freezer Storage:	-80°C for 4 months
Processed Sample:	Room temp. for 1 day
Plasma Storage:	On ice for 4 hours
5 Cycle Freeze/Thaw:	5 cycles to -70°C
Standard Solution:	6 months

Validation Results: WR 178,460 in human blood

Lower Limit of Quantitation:	0.964 ng/ml
Interday Mean, CV and RE:	0.980 ng/ml, 6.35% and +1.70%
Intraday Mean, CV and RE:	0.923 ng/ml, 9.57% and -4.28%
Standard curve range:	0.964 to 232 ng/ml
Interday Precision Concentrations:	1.93, 9.64, 57.9, and 96.5 ng/ml
CV Range:	2.90 to 7.67%
RE Range:	-4.04 to -0.0765%
Intraday Precision Concentrations:	2.04, 10.2, 61.2, and 102 ng/ml
CV Range:	3.26 to 4.33%
RE Range:	-5.34 to +0.400%
Blind Sample Assay	
Concentration Range:	1.97 to 177.5 ng/ml
RE Range:	-2.47 to +33.8%
Overall Mean Recovery:	90.4%
Stability	
Plasma Freezer Storage:	-80°C for 4 months
Processed Sample:	Room temp. for 1 day
Plasma Storage:	On ice for 4 hours
5 Cycle Freeze/Thaw:	5 cycles to -70°C
Standard Solution:	6 months

Study Description: Halofantrine and Metabolite in Human Plasma and Blood (the methodology was presented in DAMD17-92-C-2028 mid-term report)

The original version (Study Report 17 dated 4/25/90) of the silica gel stationary phase method has been modified by replacement of procainamide with WR 122,455 as internal standard and by the additional requirement that sample preparation is performed over ice.

Study Characteristics: Study Report 17B, Supplement I

Sample Assay Volume:	0.1 ml
Sample Cleanup:	Precipitate with acetonitrile

Short Validation Results: Halofantrine as free base in rat perfusate by precipitation

Lower Limit of Quantitation:	0.520 µg/ml
Interday Mean, CV and RE:	0.553 µg/ml, 2.35% and +6.35%
Intraday Mean, CV and RE:	0.502 µg/ml, 9.90% and -3.56%
Standard curve range:	0.520 to 33.28 µg/ml
Interday Precision Concentrations:	1.18, 5.90, and 2.36 µg/ml
CV Range:	1.36 to 4.09%
RE Range:	-4.72 to -0.141%
Intraday Precision Concentrations:	1.18, 5.90, and 2.36 µg/ml
CV Range:	1.60 to 3.55%
RE Range:	-5.28 to -2.90%
Overall Mean Recovery:	99.1%

Short Validation Results: WR 178460 as free base in rat perfusate by precipitation

Lower Limit of Quantitation:	0.510 µg/ml
Interday Mean, CV and RE:	0.555 µg/ml, 0.480% and +8.82%
Intraday Mean, CV and RE:	0.558 µg/ml, 8.29% and +9.38%
Standard curve range:	0.510 to 33.64 µg/ml
Interday Precision Concentrations:	1.07, 5.35, and 20.4 µg/ml
CV Range:	2.40 to 3.61%
RE Range:	-4.92 to +4.49%
Intraday Precision Concentrations:	1.07, 5.35, and 20.4 µg/ml
CV Range:	2.04 to 3.40%
RE Range:	-5.02 to +3.68%
Overall Mean Recovery:	100%
Sample Assay Volume:	0.5 ml

Study Description: Halofantrine and Metabolite in Rat Perfusate by Precipitation (the methodology was presented in DAMD17-92-C-2028 final report)

The perfusate samples were analyzed for halofantrine (1,3-dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(di-*n*-butylaminopropyl)-phenanthrene hydrochloride) and WR 178,460 (1,3 dichloro-6-trifluoromethyl-9-[1-hydroxyl-3-(N-*n*-butylamino)propyl]-phenanthrene hydrochloride) (as free bases) with an HPLC procedure that uses a silica gel column, a (methanol/water) aqueous mobile phase, a fluorescence detector, and 100 µl rat perfusate samples. Sample cleanup consisted of precipitation and centrifugation of perfusate samples with acetonitrile prior to separation by HPLC.

Assay samples were prepared by spiking known volumes of rat perfusate with a known amount (constant over all samples) of WR 122,455 (_-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol hydrochloride) internal standard (IS). Standard curve samples were generated by spiking interference free rat perfusate samples with known amounts of halofantrine and WR 178,460 (as free bases) and IS. These standard curve and assay samples were precipitated and centrifuged, then injected onto an HPLC column for separation and subsequent fluorometric detection. The peak height ratios of halofantrine and WR 178,460 (as free bases) to IS were calculated for each sample from the measured peak heights obtained by HPLC. Finally, spiked concentrations and halofantrine and WR 178,460 (as free bases) to IS peak height ratios of the standard curve samples were fit by weighted least squares linear regression to the equations for the best straight lines ($y = mx + b$, where y = peak height ratio and x = halofantrine or WR 178,460 (as free bases) concentration), and drug and metabolite concentrations in assay samples were calculated by these equations from the halofantrine and WR 178,460 (as free bases) to IS peak height ratios obtained by HPLC.

Rat perfusate samples (100 µl aliquots unless otherwise specified) to be assayed for halofantrine and WR 178,460 (as free bases) were pipetted into silanized glass culture tubes. Next, 40 µl of internal standard (WR 122,455) solution (9.90 µg/ml) was added to each and the samples vortexed. Then, 0.4 ml of acetonitrile precipitant was added to each, and the samples were vortexed for 1 min. and centrifuged for 10 min. at 3000 *g*. Each supernatant was transferred to a silanized WISP insert and injected onto the HPLC column.

By use of simple precipitation and centrifugation for sample clean-up, an unbonded silica gel column combined with an aqueous mobile phase for separation, and the superior capability of fluorescence detection, halofantrine and WR 178,460 (as free bases) can be quantitatively and reliably measured in rat perfusate samples. The assay described in the report dated November, 11, 1996, requires 100 µl rat perfusate samples to determine the concentrations in the range 0.520 - 33.28 µg/ml halofantrine (as free base) and 0.510 - 32.64 µg/ml WR 178,460 (as free base). The method involves sample cleanup with acetonitrile precipitation, separation on a silica column run with an aqueous mobile phase, and fluorescence detection. Retention times and peak shapes do not appreciably change in this particular assay within the specified injection volumes (5-20 µl) as can be seen by examination of the representative standard curve chromatograms and the corresponding standard curve (Table 1). In some assays, such changes are appreciable and can adversely affect the standard curve, requiring identical injection volumes for all samples. The LLOQs for the assay of rat perfusate, based on interday and intraday low point validation results (Table 2), has been set at 0.520 µg/ml for halofantrine and 0.510 µg/ml for WR 178,460 (as free bases). The average mean recoveries over the working range of the methodology were 99.1% for halofantrine and 100% for WR 178,460 (as free bases). The interday and intraday precision C.V.'s ranged from 1.36 to 4.09% and 1.60 to 3.55% for the method, respectively, for halofantrine and from 2.40 to 3.61% and 2.04 to 3.40% for the method, respectively, for WR 178,460 (as free bases).

Study Characteristics: Study Report 17B, Supplement II

Sample Assay Volume:	0.1 ml
Sample Cleanup:	Double extraction with methyl *t*-butyl ether

Short Validation Results: Halofantrine as free base in rat perfusate by extraction

Lower Limit of Quantitation:	10.4 ng/ml
Interday Mean, CV and RE:	11.3 ng/ml, 6.66% and +8.33%
Intraday Mean, CV and RE:	11.4 ng/ml, 6.30% and +9.78%
Standard curve range:	10.4 to 1331 ng/ml
Interday Precision Concentrations:	23.6, 70.8, 283.2, and 944 ng/ml
CV Range:	4.58 to 7.75%
RE Range:	-9.83 to -2.01%
Intraday Precision Concentrations:	23.6, 70.8, 283.2, and 944 ng/ml
CV Range:	1.49 to 7.12%
RE Range:	-5.72 to +0.140%
Overall Mean Recovery:	90.8%

Short Validation Results: WR 178460 as free base in rat perfusate by extraction

Lower Limit of Quantitation:	10.2 ng/ml
Interday Mean, CV and RE:	10.4 ng/ml, 10.7% and +1.57%
Intraday Mean, CV and RE:	10.7 ng/ml, 7.80% and +4.61%
Standard curve range:	10.2 to 1306 ng/ml
Interday Precision Concentrations:	21.4, 64.2, 257, and 856 ng/ml
CV Range:	4.13 to 7.88%
RE Range:	-4.11 to +1.78%
Intraday Precision Concentrations:	21.4, 64.2, 257, and 856 ng/ml
CV Range:	0.68 to 8.99%
RE Range:	-5.45 to +4.36%
Overall Mean Recovery:	92.4%

Study Description: Halofantrine and Metabolite in Rat Perfusate by Extraction (the methodology was presented in DAMD17-92-C-2028 final report)

The perfusate samples were analyzed for halofantrine (1,3-dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(di-*n*-butylaminopropyl)-phenanthrene hydrochloride) and WR 178,460 (as free bases) (1,3 dichloro-6-trifluoromethyl-9-[1-hydroxyl-3-(N-*n*-butylamino)propyl]-phenanthrene hydrochloride), with an HPLC procedure that uses a silica gel column, a (methanol/water) aqueous mobile phase, a fluorescence detector, and 100 µl rat perfusate samples. Sample cleanup consisted of extraction into methyl *t*-butyl ether, evaporation of extracted samples and reconstitution in methanol/0.001% HCl (4:1, v/v) prior to separation by HPLC. The method SOP contains detailed procedures and results, which are summarized below.

Assay samples were prepared by spiking known volumes of rat perfusate with a known amount (constant over all samples) of WR 122,455 (_-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol hydrochloride) internal standard (IS). Standard curve samples were generated by spiking interference free rat perfusate samples with known amounts of halofantrine and WR 178,460 (as free bases) and IS. These standard curve and assay samples were extracted, the extracts evaporated to dryness and reconstituted, then injected onto an HPLC column for separation and subsequent fluorometric detection. The peak height ratios of halofantrine and WR 178,460 (as free bases) to IS were calculated for each sample from the measured peak heights obtained by HPLC. Finally, spiked concentrations and halofantrine and WR 178,460 (as free bases) to IS peak height ratios of the standard curve samples were fit by weighted least squares linear regression to the equations for the best straight lines ($y = mx + b$, where y = peak height ratio and x = halofantrine or WR 178,460 (as free bases) concentrations), and drug and metabolite concentrations in assay samples were calculated by this equation from the halofantrine and WR 178,460 (as free bases) to IS peak height ratios obtained by HPLC.

Rat perfusate samples (100 µl aliquots unless otherwise specified) to be assayed for halofantrine and WR 178,460 (as free bases) were pipetted into 16x125 silanized glass culture tubes. Then, 100 µl water was added. Next, 20 µl of internal standard (WR 122,455) solution (1.04 µg/ml) and 50 µl of 0.1 N NaOH were added and vortexed. Then, 2 ml of methyl *t*-butyl ether was added to each, the samples were vortexed for 1 min, twice, and centrifuged for 10 min at 3000 *g*. Each samples' aqueous layer was frozen in a dry ice/methanol bath and the extraction solvent transferred to a 13x100 silanized tube. The extraction was repeated. Combined extracts were evaporated to dryness, reconstituted in 200 µl methanol/water (4:1, v/v) with a final 0.001% HCl concentration, and samples were transferred to silanized WISP inserts, and injected onto the HPLC column.

By use of a simple double extraction and centrifugation for sample clean-up, an unbonded silica gel column combined with an aqueous mobile phase for separation, and the superior capability of fluorescence detection, halofantrine and WR 178,460 (as free bases) can be quantitatively and reliably measured in rat perfusate samples. The assay described in the report dated November, 25, 1996, requires 100 µl rat perfusate samples to determine the concentrations in the range 10.4 - 1331 ng/ml halofantrine (as free base) and 10.2 - 1306 ng/ml WR 178,460 (as free base). The method involves sample extraction (double) into methyl *t*-butyl ether, separation on a silica column run with an aqueous mobile phase, and fluorescence detection. Retention times and peak shapes do not appreciably change in this particular assay within the specified injection volumes (50-100 µl) as can be seen by examination of the representative standard curve chromatograms and the corresponding standard curve (Table 1). In some assays, such changes are appreciable and can adversely affect the standard curve, requiring identical injection volumes for all samples. The LLOQs for the assay of rat perfusate, based on interday and intraday low point validation results, has been set at 10.4 ng/ml for halofantrine and 10.2 ng/ml for WR 178,460 (as free bases). The average mean recoveries over the working range of the methodology were 90.8% for halofantrine and 92.4% for WR 178,460 (as free bases). The

interday and intraday precision C.V.'s ranged from 4.58 to 7.75% and 1.49 to 7.12% for the method, respectively, for halofantrine and from 4.13 to 7.88% and 0.680 to 8.99% for the method, respectively, for WR 178,460 (as free bases). The interday and intraday precision R.E.'s ranged from -9.83 to -2.01% and -5.72 to +0.140% for the method, respectively, for halofantrine and from -4.11 to +1.78% and -5.45 to +4.36% for the method, respectively, for WR 178,460 (as free bases).

Study Characteristics: Study Report 17B, Supplement III

Sample Assay Volume:	25 µl
Sample Cleanup:	Precipitate with acetonitrile

Short Validation Results: Halofantrine as free base in rat bile by precipitation

Lower Limit of Quantitation:	0.416 µg/ml
Interday Mean, CV and RE:	0.463 µg/ml, 1.69% and +11.3%
Intraday Mean, CV and RE:	0.477 µg/ml, 9.64% and +14.6%
Standard curve range:	0.416 to 66.56 µg/ml
Interday Precision Concentrations:	0.944, 4.72, 14.16, and 28.32 µg/ml
CV Range:	2.63 to 6.85%
RE Range:	-7.59 to +2.21%
Intraday Precision Concentrations:	0.944, 4.72, 14.16, and 28.32 µg/ml
CV Range:	1.85 to 3.65%
RE Range:	-7.13 to +3.09%
Overall Mean Recovery:	104%

Short Validation Results: WR 178460 as free base in rat bile by precipitation

Lower Limit of Quantitation:	0.408 µg/ml
Interday Mean, CV and RE:	0.483 µg/ml, 3.05% and +18.4%
Intraday Mean, CV and RE:	0.468 µg/ml, 7.01% and +14.7%
Standard curve range:	0.408 to 65.28 µg/ml
Interday Precision Concentrations:	0.860, 4.30, 12.84, and 25.68 µg/ml
CV Range:	2.50 to 11.6%
RE Range:	-5.41 to +6.29%
Intraday Precision Concentrations:	0.856, 4.28, 12.84, and 25.68 µg/ml
CV Range:	1.28 to 4.12%
RE Range:	-6.07 to +1.85%
Overall Mean Recovery:	103%

Study Description: Halofantrine and Metabolite in Rat Bile by Precipitation (the methodology was presented in DAMD17-92-C-2028 final report)

Rat bile samples were analyzed for halofantrine (1,3-dichloro-6-trifluoro-methyl-9-[1-hydroxy-3-(di-*n*-butylaminopropyl)-phenanthrene hydrochloride) and WR 178,460 (as free bases) (1,3 dichloro-6-trifluoromethyl-9-[1-hydroxyl-3-(N-*n*-butylamino)propyl]-phenanthrene hydrochloride), with an HPLC procedure that uses a silica gel column, a (methanol/water) aqueous mobile phase, a fluorescence detector, and 25 µl rat bile samples. Sample cleanup consisted of precipitation and centrifugation of bile samples with acetonitrile prior to separation by HPLC. The method SOP contains detailed procedures and results, which are summarized below.

Assay samples were prepared by spiking known volumes of rat bile with a known amount (constant over all samples) of WR 122,455 (a-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol hydrochloride) internal standard

(IS). Standard curve samples were generated by spiking interference free rat bile samples with known amounts of halofantrine and WR 178,460 (as free bases) and IS. These standard curve and assay samples were precipitated and centrifuged, then injected onto an HPLC column for separation and subsequent fluorometric detection. The peak height ratios of halofantrine and WR 178,460 (as free bases) to IS were calculated for each sample from the measured peak heights obtained by HPLC. Finally, spiked concentrations and halofantrine and WR 178,460 (as free bases) to IS peak height ratios of the standard curve samples were fit by weighted least squares linear regression to the equations for the best straight lines ($y = mx + b$, where y = peak height ratio and x = halofantrine or WR 178,460 (as free bases) concentrations), and drug and metabolite concentrations in assay samples were calculated by this equation from the halofantrine and WR 178,460 (as free bases) to IS peak height ratios obtained by HPLC.

Rat bile samples (25 µl aliquots unless otherwise specified) to be assayed for halofantrine and WR 178,460 (as free bases) were pipetted into silanized glass culture tubes. Next, 10 µl of internal standard (WR 122,455) solution (9.90 µg/ml) was added and each sample was vortexed. Then, 0.2 ml of acetonitrile precipitant was added, and the samples were vortexed for 1 min. and centrifuged for 10 min. at 3000 *g*. The supernatant was transferred to a silanized WISP insert, and 20 µl was injected onto the HPLC column.

By use of simple precipitation and centrifugation for sample clean-up, an unbonded silica gel column combined with an aqueous mobile phase for separation, and the superior capability of fluorescence detection, halofantrine and WR 178,460 (as free bases) can be quantitatively and reliably measured in rat bile samples. The assay described in the report dated December, 3, 1996, requires 25 µl rat bile samples to determine the concentrations in the range 0.416 - 66.56 µg/ml halofantrine (as free base) and 0.408 - 65.28 µg/ml WR 178,460 (as free base). The method involves sample cleanup with acetonitrile precipitation, separation on a silica column run with an aqueous mobile phase, and fluorescence detection. The LLOQs for the assay of rat bile, based on interday and intraday low point validation results, has been set at 0.416 µg/ml for halofantrine and 0.408 µg/ml for WR 178,460 (as free bases). The overall mean recoveries over the working range of the methodology were 104% for halofantrine and 103% for WR 178,460 (as free bases). The interday and intraday precision C.V.'s ranged from 2.63 to 6.85% and 1.85 to 3.65% for the method, respectively, for halofantrine and from 2.50 to 11.6% and 1.28 to 4.12% for the method, respectively, for WR 178,460 (as free bases). The interday and intraday precision R.E.'s ranged from -7.59 to +2.21% and -7.13 to +3.09% for the method, respectively, for halofantrine and from -5.41 to +6.29% and -6.07 to +1.85% for the method, respectively, for WR 178,460 (as free bases).

Study Characteristics: Study Report 17B, Supplement IV

Sample Assay Volume:	0.1 ml
Sample Cleanup:	Double extraction with methyl *t*-butyl ether

Short Validation Results: Halofantrine as free base in rat bile by extraction

Lower Limit of Quantitation:	20.8 ng/ml
Interday Mean, CV and RE:	22.8 ng/ml, 0.62% and +9.62%
Intraday Mean, CV and RE:	23.0 ng/ml, 4.19% and +10.5%
Standard curve range:	20.8 to 1331 ng/ml
Interday Precision Concentrations:	47.2, 94.4, 378, and 944 ng/ml
CV Range:	3.57 to 15.5%
RE Range:	-9.56 to +1.52%
Intraday Precision Concentrations:	47.2, 94.4, 378, and 944 ng/ml
CV Range:	3.82 to 13.4%
RE Range:	-10.3 to -1.45%
Overall Mean Recovery:	70.5%

Short Validation Results: WR 178460 as free base in rat bile by extraction

Lower Limit of Quantitation:	20.4 ng/ml
Interday Mean, CV and RE:	22.6 ng/ml, 7.84% and +10.5%
Intraday Mean, CV and RE:	20.5 ng/ml, 4.63% and +0.69%
Standard curve range:	20.4 to 1306 ng/ml
Interday Precision Concentrations:	42.8, 85.6, 342, and 856 ng/ml
CV Range:	1.70 to 17.0%
RE Range:	-5.67 to -1.01%
Intraday Precision Concentrations:	42.8, 85.6, 342, and 856 ng/ml
CV Range:	2.63 to 11.5%
RE Range:	-3.37 to +4.75%
Overall Mean Recovery:	90.1%

Study Description: Halofantrine and Metabolite in Rat Bile by Extraction (the methodology was presented in DAMD17-92-C-2028 final report)

The bile samples were analyzed for halofantrine (1,3-dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(di-*n*-butylaminopropyl)-phenanthrene hydrochloride) and WR 178,460 (as free bases) (1,3 dichloro-6-trifluoromethyl-9-[1-hydroxyl-3-(N-*n*-butylamino)propyl]-phenanthrene hydrochloride), with an HPLC procedure that uses a silica gel column, a (methanol/water) aqueous mobile phase, a fluorescence detector, and 100 µl rat bile samples. Sample cleanup consisted of extraction into methyl *t*-butyl ether, evaporation of extracted samples and reconstitution in methanol/0.001% HCl (4:1, v/v) prior to separation by HPLC. The method SOP contains detailed procedures and results, which are summarized below.

Assay samples were prepared by spiking known volumes of rat bile with a known amount (constant over all samples) of WR 122,455 (a-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol hydrochloride) internal standard (IS). Standard curve samples were generated by spiking interference free rat bile samples with known amounts of halofantrine and WR 178,460 (as free bases) and IS. These standard curve and assay samples were extracted, the extracts evaporated to dryness and reconstituted, then injected onto an HPLC column for separation and subsequent fluorometric detection. The peak height ratios of halofantrine and WR 178,460 (as free bases) to IS were calculated for each sample from the measured peak heights obtained by HPLC. Finally, spiked concentrations and halofantrine and WR 178,460 (as free bases) to IS peak height ratios of the standard curve samples were fit by weighted least squares linear regression to the equations for the best straight lines ($y = mx + b$, where y = peak height ratio and x = halofantrine or WR 178,460 (as free bases) concentrations), and drug and metabolite concentrations in assay samples were calculated by this equation from the halofantrine and WR 178,460 (as free bases) to IS peak height ratios obtained by HPLC.

Rat bile samples (100 µl aliquots unless otherwise specified) to be assayed for halofantrine and WR 178,460 (as free bases) were pipetted into 16x125 silanized glass culture tubes on ice. Then, 100 µl of water was added. Next, 20 µl of internal standard (WR 122,455) solution (1.04 µg/ml) and 50 µl of 0.1 N NaOH were added and vortexed. Then, 2 ml of methyl *t*-butyl ether was added to each, and the samples were vortexed for 1 min., twice, and centrifuged for 10 min. at 3000 *g*. Each samples aqueous layer was frozen in a dry ice/methanol bath and the extraction solvent transferred to a 13x100 silanized tube. The extraction was repeated. Combined extracts were evaporated to dryness, reconstituted in 200 µl methanol/water (4:1, v/v) with a final 0.001% HCl concentration, and samples were transferred to silanized WISP inserts, and injected onto the HPLC column.

By use of a simple double extraction and centrifugation for sample clean-up, an unbonded silica gel column combined with an aqueous mobile phase for separation, and the superior capability of fluorescence detection, halofantrine and WR 178,460 (as free bases) can be quantitatively and reliably measured in rat bile samples. The assay described in the report dated December, 5, 1996, requires 100 µl rat bile samples to determine the concentrations in the range 20.8 - 1331 ng/ml halofantrine (as free base) and 20.4 - 1306 ng/ml WR 178,460 (as free base). The method involves sample extraction (double) into methyl *t*-butyl ether, separation on a silica column run with an aqueous mobile phase, and fluorescence detection. Retention times and peak shapes do not appreciably change in this particular assay within the specified injection volumes (50-100 µl) as can be seen by examination of the representative standard curve chromatograms and the corresponding standard curve (Table 1). In some assays, such changes are appreciable and can adversely affect the standard curve, requiring identical injection volumes for all samples. The LLOQs for the assay of rat bile, based on interday and intraday low point validation results, has been set at 20.8 ng/ml for halofantrine and 20.4 ng/ml for WR 178,460 (as free bases). The overall average recoveries over the working range of the methodology were 70.5% for halofantrine and 90.1% for WR 178,460 (as free bases). The interday

and intraday precision C.V.'s ranged from 3.57 to 15.5% and 3.82 to 13.4% for the method, respectively, for halofantrine and from 1.70 to 17.0% and 2.63 to 11.5% for the method, respectively, for WR 178,460 (as free bases). The interday and intraday precision R.E.'s ranged from -9.56 to +1.52% and -10.3 to -1.45% for the method, respectively, for halofantrine and from -5.67 to -1.01% and -3.37 to +4.75% for the method, respectively, for WR 178,460 (as free bases).

Study Characteristics: Study Report 17B, Supplement V

Sample Assay Volume:	0.200 ml
Sample Cleanup:	Precipitate with acetonitrile

Short Validation Results: Halofantrine as free base in rat liver homogenate

Lower Limit of Quantitation:	0.540 µg/ml
Interday Mean, CV and RE:	0.584 µg/ml, 9.59% and +8.21%
Intraday Mean, CV and RE:	0.550 µg/ml, 9.30% and +1.76%
Standard curve range:	0.540 to 69.1 µg/ml
Interday Precision Concentrations:	1.07, 5.35, and 24.6 µg/ml
CV Range:	2.52 to 5.45%
RE Range:	+0.187 to +5.62%
Intraday Precision Concentrations:	1.07, 5.35, and 24.6 µg/ml
CV Range:	2.33 to 4.86%
RE Range:	-0.685 to +10.4%
Overall Mean Recovery:	99.1%

Short Validation Results: WR 178460 as free base in rat liver homogenate

Lower Limit of Quantitation:	0.540 µg/ml
Interday Mean, CV and RE:	0.559 µg/ml, 9.11% and +3.58%
Intraday Mean, CV and RE:	0.563 µg/ml, 7.88% and +4.32%
Standard curve range:	0.540 to 69.1 µg/ml
Interday Precision Concentrations:	1.07, 5.35, and 24.6 µg/ml
CV Range:	2.30 to 8.55%
RE Range:	-3.93 to +4.70%
Intraday Precision Concentrations:	1.07, 5.35, and 24.6 µg/ml
CV Range:	2.53 to 5.25%
RE Range:	-2.78 to +5.76%
Overall Mean Recovery:	94.6%

Study Description: Halofantrine and Metabolite in Rat Liver Homogenate (the methodology was presented in DAMD17-92-C-2028 final report)

Rat liver samples were analyzed for halofantrine (1,3-dichloro-6-trifluoromethyl-9-[1-hydroxy-3-(di-*n*-butylaminopropyl)-phenanthrene hydrochloride) and WR 178,460 (as free bases) (1,3 dichloro-6-trifluoromethyl-9-[1-hydroxyl-3-(N-*n*-butylamino)propyl]-phenanthrene hydrochloride), with an HPLC procedure that uses a silica gel column, a (methanol/water) aqueous mobile phase, a fluorescence detector, and 200 µl rat liver homogenate samples. The method SOP contains detailed procedures and results, which are summarized below.

Assay samples were prepared by spiking known volumes of rat liver homogenate with a known amount (constant over all samples) of WR 122,455 (a-(2-piperidyl)-3,6-bis(trifluoromethyl)-9-phenanthrenemethanol hydrochloride) internal standard (IS). Liver homogenate is produced by homogenizing 1 g liver

in 5 ml of a methanol/water (with 1% final HCl concentration) buffer in a blender. Standard curve samples were generated by spiking interference free rat liver homogenate samples with known amounts of halofantrine and WR 178,460 (as free bases) and IS. These standard curve and assay samples were precipitated and centrifuged, then injected onto an HPLC column for separation and subsequent fluorometric detection. The peak height ratios of halofantrine and WR 178,460 (as free bases) to IS were calculated for each sample from the measured peak heights obtained by HPLC. Finally, spiked concentrations and halofantrine and WR 178,460 (as free bases) to IS peak height ratios of the standard curve samples were fit by weighted least squares linear regression to the equations for the best straight lines ($y = mx + b$, where y = peak height ratio and x = halofantrine or WR 178,460 (as free bases) concentrations), and drug and metabolite concentrations in assay samples were calculated by this equation from the halofantrine and WR 178,460 (as free bases) to IS peak height ratios obtained by HPLC.

Rat liver homogenate samples (200 µl aliquots unless otherwise specified) to be assayed for halofantrine and WR 178,460 (as free bases) were pipetted into silanized glass culture tubes. Then, 0.2 ml of acetonitrile precipitant was added, and the samples were vortexed for 1 min. Next, 40 µl of internal standard solution (9.90 µg/ml WR 122,455) was added and each sample was vortexed and centrifuged for 10 min. at 3000 *g*. The supernatant was transferred to a silanized WISP insert, and 5 µl was injected onto the HPLC column.

By use of simple precipitation and centrifugation for sample clean-up, an unbonded silica gel column combined with an aqueous mobile phase for separation, and the superior capability of fluorescence detection, halofantrine and WR 178,460 (as free bases) can be quantitatively and reliably measured in rat liver homogenate samples. The assay described in the report dated January, 28, 1997, requires 200 µl rat liver homogenate samples to determine the concentrations in the range 0.540 - 69.1 µg/ml halofantrine and WR 178,460 (as free bases). The method involves sample cleanup with acetonitrile precipitation, separation on a silica column run with an aqueous mobile phase, and fluorescence detection. The LLOQs for the assay of rat liver homogenate, based on interday and intraday low point validation results, have been set at 0.540 µg/ml for halofantrine and 0.540 µg/ml for WR 178,460 (as free bases). The overall mean recoveries over the working range of the methodology were 99.1% for halofantrine and 94.6% for WR 178,460 (as free bases). The result ranges for halofantrine interday were C.V. 2.52 to 5.45% and R.E. +0.514 to +5.62%. The ranges for halofantrine intraday were C.V. 2.33 to 4.86% and R.E. -0.685 to +10.4%. The ranges for WR 178,460 interday were C.V. 2.30 to 8.55% and R.E. -3.93 to +4.70%. The ranges for WR 178,460 intraday were C.V. 2.53 to 5.25% and R.E. -2.78 to +5.76%.

Study Report 18: WR 6026 and Metabolite in Human Plasma and Blood

Study Characteristics: Study Report 18

Test Article:	WR 6026, WR 211,789
Test System:	human plasma and blood
Internal Standard:	chlorpheniramine
Sample Assay Volume:	0.5 ml
Sample Cleanup:	methyl *t*-butyl ether extraction

Analytical System

Detector:	UV at 263 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5μ particle size
Mobile Phase:	acetonitrile/water (3:2, v/v) final concentration of 5 mM $(NH_4)_2HPO_4$ at pH 8.8

Validation Results: WR 6026 in human plasma

Quantitation Limit:	0.980 ng/ml
Standard curve range:	0.980-98.0 ng/ml
Interday Precision	
Concentration Range:	2.06-77.3 ng/ml
CV Range:	3.05-6.82%
Intraday Precision	
Concentration Range:	2.06-77.3 ng/ml
CV Range:	3.22-9.39%
Blind Sample Assay	see Appendix A, DAMD17-92-C-2028 Midterm Report
Mean Recovery:	74.5%
Stable Plasma Storage:	-20°C for 3 months

Validation Results: WR 211,789 in human plasma

Quantitation Limit:	1.21 ng/ml
Standard curve range:	1.21-121 ng/ml
Interday Precision	
Concentration Range:	2.14-80.1 ng/ml
CV Range:	5.19-8.98%
Intraday Precision	
Concentration Range:	2.14-80.1 ng/ml
CV Range:	4.42-7.86%
Blind Sample Assay	see Appendix A, DAMD17-92-C-2028 Midterm Report
Mean Recovery:	93.8%

Validation Results: WR 6026 in human blood

Quantitation Limit:	0.980 ng/ml
Standard curve range:	0.980-98.0 ng/ml
Interday Precision	
Concentration Range:	1.96-78.4 ng/ml
CV Range:	1.56-6.38%
Intraday Precision	
Concentration Range:	1.96-78.4 ng/ml
CV Range:	2.31-5.36%
Stable Plasma Storage:	-20°C for 1 month -70°C for 3 months

Validation Results: WR 211,789 in human blood

Quantitation Limit:	1.21 ng/ml
Standard curve range:	1.21-121 ng/ml
Interday Precision	
Concentration Range:	2.40-96.0 ng/ml
CV Range:	1.74-5.12%
Intraday Precision	
Concentration Range:	2.40-96.0 ng/ml
CV Range:	1.76-4.85%

Study Description: WR 6026 and Metabolite in Human Plasma and Blood (the methodology was presented in DAMD17-92-C-2028 mid-term report)

Sets of blind plasma and blood samples, prepared April 1, 1993, were received. Blind plasma sample results were enclosed with Quarterly Report 8. Upon analysis of blood samples, results will be forwarded to the COR. Acceptable results will be incorporated into Study Report 18, "Quantitation of WR 6026 and WR 211,789 (as Free Bases) in Plasma and Blood by High-Performance Liquid Chromatography." The test of stability is in progress.
Procedures Required to Complete Validation

The following list details changes that were instituted for plasma sample analysis, but that have not been tested for validation of the blood sample analytical method.

1. Following addition of 5 ml of methyl-*t*-butyl ether, vortex [not rotate] samples for 1 [not 15] min.
2. Adjust the mobile phase pH to 8.8 [not 7.0].
3. Stock and working solutions were stored at -20°C [not 4°C].

The following list details validation tests that have not been done.

1. Stability of WR 211,789 (free base) at -80°C and -20°C in blood and plasma.
2. Recovery of WR 6026 and WR 211,789 from blood.
3. Precision of WR 6026 and WR 211,789 (as free bases) in blood with mobile phase pH = 8.8, storage of stock and working solutions at -20°C, and vortexing extraction samples for 1 min.
4. Accuracy for WR 6026 and WR 211,789 (as free bases) in plasma and blood on blind spiked samples prepared by the Walter Reed Army Institute of Research.
5. Interference: To determine whether known compounds would interfere with detection of WR 6026 or WR 211,789 (as free bases), the retention times relative to CPA in mobile phase of several WR 6026 (free base) analogs could include WR 225,742 and WR 254,421 (free base).

Study Description

WR 6026 (dihydrochloride) (6-methoxy-8-(6-diethyl amino hexyl amino) lepidine dihydrochloride) (see figure below), is a very effective antileishmanial drug in hamsters infected with *Leishmania donovani* .[16]

Because antimony compounds are not always effective and the other drugs in use have toxic effects,[17,18] alternative therapies are needed. Since WR 6026 (dihydrochloride) is a likely candidate and since WR 6026 (dihydro-chloride) is scheduled for clinical testing in the near future, it is extremely important to develop an analytical method capable of measuring concentra-tions of WR 6026 (free base) at nanogram per milliliter concentrations in biological samples.

This report describes an assay developed to determine the concentrations of WR 6026 and of its mono dealkylated metabolite, WR 211,789, (as free bases) in blood and plasma. This new assay provides significant improvements over capabilities of earlier assays with increased sensitivity for the detection of WR 6026 (free base)[19]and inclusion of WR 211,789 (free base) in the methodology (Study Report 10).

Plasma samples (0.5 ml transferred with a plastic tipped pipetter to silanized culture tubes (see SOP #3-11 for silanization procedure)) were vortexed with 100 µl of a 1.00 µg/ml chlorpheniramine maleate internal standard working solution and 100 µl of a 1 N NaOH solution for 10 s. Next, 5 ml of methyl-*t*-butyl ether was added and samples were vortexed for 1 min, then centrifuged for 10 min at 3000 *g*. Then, for each sample, the aqueous layer was frozen in a dry ice/methanol bath and the organic layer were decanted into a new silanized culture tube. Finally, the sample's organic layer was evaporated to dryness under prepurified nitrogen, reconstituted in 200 µl of mobile phase, vortexed for 1 min, transferred to silanized WISP inserts, and injected onto the HPLC column.

Blood samples (0.5 ml transferred with a plastic tipped pipetter to silanized culture tubes) were vortexed for 1 min with 0.5 ml of nanopure water, and the mixtures were sonicated for 10 min. Then, these samples were prepared like

plasma samples beginning with addition of 100 µl of the internal standard working solution.

No degradation of WR 6026 (free base) in plasma frozen at -20°C or blood frozen at -80°C was seen for the duration of the stability study. However, noticeable degradation of WR 6026 (free base) in blood frozen at -20°C was observed by the third month at all concentrations.

Two standard curves for each assay were constructed from the chromatographic data; a low range curve from the 0 to 14.7 ng/ml for WR 6026 and 0 to 18.1 ng/ml for WR 211,789 standard curve samples and a high range curve from the 0 to 98.0 ng/ml for WR 6026 and 0 to 121 ng/ml (i.e. all) standard curve samples in order to obtain more accurate determinations of low level WR 6026 and WR 211,789 (free base) concentrations. The low range standard curve was used to calculate drug or metabolite concentrations for assayed samples when the peak height ratio of the sample was less than or equal to the calculated peak height ratio at the highest concentration of the low range curve (as calculated from the low range curve). The high range curve was used to calculate results for samples with peak height ratios greater than the calculated peak height ratio at the highest concentration of the low range curve (as calculated from the low range curve).

Typical plasma and blood chromatograms show WR 6026 (free base), WR 211,789 (free base) and internal standard, chlorpheniramine, peaks that are baseline separated and separated from other components of the sample.

A linear relationships was demonstrated between the WR 6026 and WR 211,789 (free base) spiked concentrations to the WR 6026 and WR 211,789 (free base) to internal standard peak height ratios for the plasma and blood assays. Linear regression analysis of concentration versus the peak height ratio gave coefficients of determination (r^2) of 0.989 or better for these typical standard curves. The linear range of the standard curves covered WR 6026 (free base) concentrations in plasma and blood in the range 0.980 to 98.0 ng/ml and WR 211,789 (free base) concentrations in plasma and blood in the range 1.21 to 121 ng/ml. The reversed-phase system (alkyl bonded silica gel with an aqueous mobile phase) is the most widely used HPLC technique in assays for drugs in biological fluids. In this kind of a system, the retention mechanism depends mainly on the lipophilic character of substances to be analyzed. Such a mechanism also retains considerable amounts of other lipophilic substances, thereby interfering with the drug peak. On the other hand, in a system consisting of a bare silica gel and an aqueous mobile phase, the retention mechanism results mainly from ion exchange[20] and only partially from lipophilic interactions. Thus, endogenous non-ionic neutral lipid compounds and anionic compounds will not be retained on the silica gel column; only the cationic (e.g. ammonium) ions will be retained. The interfering substances in biological fluids elute at the solvent front, leaving a very clean baseline around the retention time of the drug.

Validation trials in our laboratory for an earlier study (Study Report 10) were undertaken to include in the WR 6026 (free base) assay the capability to measure WR 211,789 (free base), a mono dealkylated metabolite of WR 6026 (free base), concentrations in biological samples. Large variations between spiked and recovered concentrations were observed in that study. Although WR 211,789 has been detected in a rat microsomal preparation,[21] it has not been detected in plasma in human studies, perhaps because the detection limit of the assay used was only 10 ng/ml.[19] WR 211,789 plasma standard curves in the trials were of higher quality than blood standard curves. The current report describes an adaptation of the WR 6026 (free base) methodology or a modification of the methodology presented in the earlier report (Study Report 10), in which a 5 to 10 fold increase in sensitivity has been gained that makes detection of WR 211,789 (free base) in human plasma possible at higher WR 6026 (dihydrochloride) doses.

In addition, compared to an even earlier methodology,[19] the WR 6026 (free base) HPLC method presented here offers increased sensitivity and extends the range of biological fluids that can be assayed. The earlier method measured WR 6026 (free base) in plasma cleaned by protein precipitation (with acetonitrile) and column elution (from a C2 extraction column), had a 6.44 ng/ml WR 6026 (free base) detection limit, used WR 223,658 as an internal standard, required a C8 bonded silica gel HPLC column, used a 60:40 (v/v) acetonitrile/water mobile phase at pH 5.5 with 0.2% final concentrations of SDS and glacial acetic acid, and measured WR 211,789 (free base) with a minimum detection limit of 8 ng/ml. The newer method measures WR 6026 (free base) in plasma and blood cleaned by extraction with 99:1 (v/v) pentane/acetonitrile, has a 0.980 ng/ml WR 6026 (free base) detection limit, uses chlorpheniramine maleate as an internal standard, requires an unbonded silica gel HPLC column, uses a 70:30 (v/v) acetonitrile/water mobile phase at pH 7.0 with 5 mM final concentration of dibasic ammonium phosphate, but could not measure WR 211,789 (free base) with a minimum detection limit much better than 8 ng/ml. The current modified method measures WR 6026 and WR 211,789 (free base) in plasma and blood cleaned by extraction with methyl-*t*-butyl ether, has 0.980 ng/ml WR 6026 and 1.21 ng/ml WR 211,789 (as free bases) detection limits, uses chlorpheniramine maleate as an internal standard, requires an unbonded silica gel HPLC column, uses a 60:40 (v/v) acetonitrile/water mobile phase at pH 8.8 with 5 mM final concentration of dibasic ammonium phosphate.

HPLC assays for basic amine drugs in biological samples that make use of a silica gel column and an aqueous mobile phase have been operated in this laboratory for over 5 years.[22,23,24] In the WR 6026 (free base) HPLC method presented here, the use of an unbonded silica gel column, an aqueous mobile phase, and UV detection at 263 nm yields satisfactory results for the determination of WR 6026 and WR 211,789 (as free bases) in (0.5 ml) plasma and blood samples. The method is simple in that a single extraction step and evaporation of solvent prior to injection are required. Recovery of WR 6026 (free base) averaged 74.5%, while recovery of WR 211,789 (free base) averaged 93.8% from plasma. The minimum quantitation limits of the assay were 0.980 ng/ml for WR 6026 (free base) and 1.21 ng/ml for WR 211,789 (free base) for blood and plasma. The coefficients of variation of the inter- and intraday assay precision analyses

were less than 10% at all concentrations. The method is simple, precise, more sensitive, and includes the capability of quantitating WR 211,789 (free base) as well as the parent drug compared to earlier methods.

Study Report 19: Mefloquine in Human Blood

Study Characteristics: Study Report 19

Test Article:	Mefloquine
Test System:	human blood
Internal Standard:	chlorpheniramine
Sample Assay Volume:	0.5 ml
Sample Cleanup:	pentane/methylene chloride (7:3, v/v) extraction

Analytical System

Detector:	UV at 280 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5µ particle size
Mobile Phase:	methanol/water (4:1, v/v) final concentration of 5 mM $(NH_4)_2HPO_4$ at pH 7.5

Validation Results: Mefloquine in blood

Quantitation Limit:	7.36 ng/ml
Standard curve range:	7.36-2210 ng/ml
Interday Precision	
Concentration Range:	14.7-1472 ng/ml
CV Range:	3.94-8.41%
Intraday Precision	
Concentration Range:	14.7-1472 ng/ml
CV Range:	2.74-10.9%
Blind Sample Assay	
Concentration Range:	11.52-1536 ng/ml
Bias Range:	-12.6 to +7.20%
Mean Recovery:	91.5%
Stable Blood Storage:	-20°C for 4 months

Study Description: Mefloquine in Human Blood (the methodology was presented in DAMD17-92-C-2028 mid-term report)

Mefloquine (hydrochloride), (WR 142,490: erythro-a-(2-piperidyl)-2,8-bis(trifluoromethyl)-4-quinolinemethanol hydrochloride), is an alternative curative agent for the treatment of falciparum malaria.[25,26] Mefloquine (hydrochloride) has also been shown to prophylactically suppress mosquito induced infections by *Plasmodium vivax* and *P. falciparum* in human volunteers.[27,28] Published plasma and/or blood analytical methods employ gas-liquid chromatography (GLC)[29,30,31] thin layer chromatography (TLC),[32] gas chromatography-mass spectrometry (GC-MS)[33] or high performance liquid chromatography (HPLC) (Study Reports 6 and 14).[34,35,36,37,38,39,40] The GLC

methods require derivitization, and sample volume in the method described by Nakagawa, *et al.* uses 5 ml samples. The TLC method has no internal standard and is insufficiently sensitive. The GC-MS method requires derivitization and the increased expense of mass spectrometry.

Many HPLC have been reported. The method reported by Grindel, *et al.*, required three times extraction from 5 ml plasma samples and, upon solvent evaporation, the residues required overnight storage in a vacuum desiccator. Kapetanovic, *et al.* used a 3 step extraction of 1 ml samples. Our earlier study (Study Report 6) described a protein precipitation method for 0.2 ml plasma samples. Franssen, *et al.*, described a method for plasma and blood analysis for mefloquine and its carboxylic acid metabolite with 50 ng/ml mefloquine and 100 ng/ml metabolite detection limits. Karbwang, *et al.*, described a 50 ng/ml detection limit, 100 ng/ml quantitation limit method for mefloquine in blood and plasma. Coleman, *et al.*, measured mefloquine at 10 ng/ml in liver perfusate. Riviere *et al.*, presented a method with a 20 ng/ml detection limit and 100 ng/ml quantitation limit in plasma. Bergqvist, *et al.*, describe two HPLC methods for determination of mefloquine and its principal metabolite in plasma and blood, the first with 30 ng/ml plasma and 150 ng/ml blood quantitation limits and the second with 75 ng/ml quantitation limits for both compounds.

We reported (Study Report 14) the development of a simple and rapid HPLC assay for mefloquine (free base) that requires 0.5 ml plasma samples and a one step extraction, has an 7.36 ng/ml quantitation limit and produces chromatograms with a cleaner baseline than our previous method. Study Report 19 describes the extension of our plasma method to include analysis of blood samples. Study Report 19 also describes status of steps taken toward extension of the method for determination of the main mefloquine metabolite, WR 160,972 (2,8-bis-(trifluoromethyl)-4-quinoline carboxylic acid).[41]

The blood method was modified from the plasma method described in Study Report 14, "Quantitation of Mefloquine (Free Base) in Plasma by High-Performance Liquid Chromatography, Extraction Method." The blood method primarily differs from the plasma method in sample preparation by:

1. Allowing blood standard curve calibrator samples to equilibrate for 1 hour following spiking with mefloquine working solutions;

2. Addition of 0.5 ml water; and

3. Sonication for 10 min prior to addition of internal standard.

Blood samples for analysis are pipetted (0.5 ml) into screw top tubes. Add 100 µl of a saturated solution of sodium carbonate and vortex the mixture for 1 min. Then, add 100 µl of the internal standard working solution (CPA, 12 µg/ml) and vortex the mixture for 1 min. The sample is extracted with 5 ml of pentane/methylene chloride (7:3, v/v), evaporated to dryness under nitrogen, resuspended in 200 µl of mobile phase and injected (40-80 µl) onto the HPLC column.

An addendum with blind sample results enclosed with Quarterly Report 6 completed Study Report 19 (Status Report, dated January 14, 1992 and titled "Quantitation of Mefloquine (Free Base) in Blood by High-Performance Liquid Chromatography, Extraction Method." Further work on this assay is scheduled to include WR 160,972 method development, but work on this aspect of the project has been assigned a low priority by the COR.

Study Report 21: *p*-Aminoheptanophenone and Metabolites in Dog Plasma and Rat Plasma

Study Characteristics: Study Report 21	
Test Article:	WR 269,410 (*p*-aminoheptanophenone)
Test System:	dog plasma
Internal Standard:	WR 258,948 (*p*-aminooctanophenone)
Sample Assay Volume:	0.5 ml
Sample Cleanup:	methyl *t*-butyl ether extraction
Analytical System	
Detector:	UV at 316 nm
Column Type:	C18 bonded silica
Column Size:	4.6x250 mm, 5µ particle size
Mobile Phase:	acetonitrile/water (1:1, v/v) and 0.15% H_3PO_4
Validation Results:	
Quantitation Limit:	4.08 ng/ml
Standard curve range:	4.08-816 ng/ml
Blind Sample Assay	see Appendix A, DAMD17-92-C-2028 Midterm Report

Study Description: *p*-Aminoheptanophenone and Metabolites in Dog Plasma and Rat Plasma (the analytical procedure was presented in DAMD17-92-C-2028 final report)

Method validation will be reported in Study Report 21 (preparation in progress covering *p*-aminoheptanophenone (PAHP, WR 269,410) *p*-aminooctanophenone (PAOP, WR 258,948) and *p*-aminopropiophenone (PAPP, WR 302)). Results from the analysis of blind spiked (by WRAIR) dog plasma samples were presented in Appendix A of the mid-term report.

Study Report 22: WR 6026 and Metabolites in Human Urine

Study Characteristics: Study Report 22

Test Article:	WR 6026 WR 211,789 WR 254,421
Test System:	human urine
Internal Standard:	verapamil
Sample Assay Volume:	0.5 ml
Sample Cleanup:	methyl *t*-butyl ether extraction

Analytical System

Detector:	UV at 350 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5µ particle size
Mobile Phase:	acetonitrile/0.0075% phosphoric acid (80:20, v/v) at pH 6.9.

Validation Results WR 6026 free base

Quantitation Limit:	5.17 ng/ml
Interday CV:	14.8%
Interday Error:	2.44%
Standard curve range:	2.17-414 ng/ml
Interday Precision	
Concentration Range:	10.4-259 ng/ml
CV Range:	3.90-7.42%
Intraday Precision	
Concentration Range:	10.4-259 ng/ml
CV Range:	3.83-28.4%
Blind Sample Assay	
Concentration Range:	5.20-101.2 ng/ml
Bias Range:	-10.6 to +33.9%
Mean Recovery:	97.2%
Stable Plasma Storage:	-70°C for 4 months
Stable Prepared Sample:	Room temp. for 48 hours

Validation Results WR 211,789 free base

Quantitation Limit:	509 ng/ml
Interday CV:	14.7%
Interday Error:	4.55%
Standard curve range:	5.09-407 ng/ml
Interday Precision	
Concentration Range:	10.2-255 ng/ml
CV Range:	4.07-10.0%
Intraday Precision	
Concentration Range:	10.2-255 ng/ml
CV Range:	5.12-23.3%
Blind Sample Assay	
Concentration Range:	5.20-102.6 ng/ml
Bias Range:	-11.8 to +33.9%
Mean Recovery:	92.8%
Stable Plasma Storage:	-70°C for 4 months
Stable Prepared Sample:	Room temp. for 48 hours

Validation Results WR 254,421 free base

Quantitation Limit:	45.4 ng/ml
Interday CV:	7.51%
Interday Error:	1.60%
Standard curve range:	45.4-3630 ng/ml
Interday Precision	
Concentration Range:	90.8-2270 ng/ml
CV Range:	3.09-5.86%
Intraday Precision	
Concentration Range:	90.8-2270 ng/ml
CV Range:	3.55-10.0%
Blind Sample Assay	
Concentration Range:	50.1-979.4 ng/ml
Bias Range:	-10.7 to +9.63%
Mean Recovery:	94.2%
Stable Plasma Storage:	-70°C for 4 months
Stable Prepared Sample:	Room temp. for 48 hours

Study Description: WR 6026 and Metabolites in Human Urine (the methodology was presented in DAMD17-92-C-2028 final report)

Study Report 22 "Quantitation of WR 6026, WR 211,789 and WR 254,421 (as Free Bases) in Human Urine by High Performance Liquid Chromatography,"

was submitted for review July 18, 1994. This method is a modified version of the plasma method.

WR 6026 (dihydrochloride) (6-methoxy-8-(6-diethyl amino hexyl amino) lepidine dihydrochloride) (Figure 1), is a very effective antileishmanial drug in hamsters infected with *Leishmania donovani* .[16] Because antimony compounds are not always effective and the other drugs in use have toxic effects,[17,18] alternative therapies are needed. Since WR 6026 (dihydrochloride) is a likely candidate and since WR 6026 (dihydro-chloride) is scheduled for clinical testing, it is extremely important to develop an analytical method capable of measuring concentrations of WR 6026 (free base) at nanogram per milliliter concentrations in biological samples. This report describes an assay developed to determine the concentrations (as free bases) of WR 6026 and of its metabolites, WR 211,789 (6-methoxy-8-(6-ethyl-aminohexylamino) lepidine dihydrochloride, hemihydrate) and WR 254,421 (8-(6'-N,N-diethylaminohexyl)amino-4-hydroxymethyl-6-methoxyquinoline, dihydrochloride) in urine. WR 211,789 has been detected in a rat microsomal preparation.[21] This assay adds the capability of detection of WR 6026, WR 211,789 and WR 254,421 (as free bases) in urine to earlier assays for WR 6026 and WR 211,789 in plasma and blood.

Assay samples were prepared by spiking known volumes of human urine with a known amount (constant over all samples) of the verapamil internal standard (IS). Standard curve samples were generated by spiking interference free human urine samples with known amounts of WR 6026, WR 211,789 and WR 254,421 (as free bases) and IS. These standard curve and assay samples were extracted, then injected onto an HPLC column for separation and subsequent ultraviolet detection. The peak height ratios of WR 6026, WR 211,789 and WR 254,421 (as free bases) to IS were calculated for each sample from the measured peak heights obtained by HPLC. Finally, standard curve concentrations and WR 6026, WR 211,789 and WR 254,421 (as free bases) to IS peak height ratios of the standard curve samples were fit by least squares linear regression to the equation for the best straight line ($y = mx + b$, where y = peak height ratio and x = WR 6026, WR 211,789 or WR 254,421 (as free bases) concentrations), and drug concentrations in assay samples were calculated by this equation from the WR 6026, WR 211,789 and WR 254,421 (as free bases) to IS peak height ratios obtained by HPLC.

Sample volume taken for analysis was 0.5 ml of urine. A constant amount, approximately 5 μg, of the internal standard, verapamil, was added to and mixed with each sample. Next, 100 μl of 1 N NaOH was added to and mixed with each sample. Then, samples were extracted with 5 ml of methyl *t*-butyl ether. The extraction solution was transferred to a second culture tube, evaporated to dryness under nitrogen, and reconstituted in 200 μl of mobile phase. Finally 20-160 μl of the sample was injected onto the HPLC column.

In typical chromatograms for blank urine and urine samples spiked with WR 6026, WR 211,789 or WR 254,421, WR 6026, WR 211,789 or WR 254,421 eluted at 15.3, 14.3, and 18.2 minutes, respectively, and the internal standard eluted at 12.4

minutes. The coefficients of determination for WR 6026, WR 211,789 or WR 254,421 interday and intraday precision standard curves were 0.9825 or higher.

The reversed-phase system (alkyl bonded silica gel with an aqueous mobile phase) is the most widely used HPLC technique in assays for drugs in biological fluids. In this kind of a system, the retention mechanism depends mainly on the lipophilic character of substances to be analyzed. Such a mechanism also retains considerable amounts of other lipophilic substances, thereby interfering with the drug peak. On the other hand, in a system consisting of a bare silica gel and an aqueous mobile phase, the retention mechanism results mainly from ion exchange and only partially from lipophilic interactions. Thus, endogenous non-ionic neutral lipid compounds and anionic compounds will not be retained on the silica gel column; only the cationic (e.g. ammonium) ions will be retained. The interfering substances in biological fluids elute at the solvent front, leaving a very clean baseline around the retention time of the drug.

HPLC assays for basic amine drugs in biological samples that make use of a silica gel column and an aqueous mobile phase have been operated in this laboratory for over 5 years. By use of an organic solvent extraction step for sample clean-up, an unbonded silica gel column combined with an aqueous mobile phase for separation, and the superior capability of ultraviolet detection, the free base concentrations of WR 6026, WR 211,789 and WR 254,421 can be quantitatively and reliably measured in human urine samples. The assay described in the report dated July, 18, 1994, requires 0.5 ml urine samples to determine the free base concentrations of WR 6026, WR 211,789 or WR 254,421. The method involves sample cleanup with a methyl *t*-butyl ether extraction, separation on an unbonded silica gel column (5 µm particle size) run with an aqueous mobile phase, and ultraviolet detection. The minimum quantitation limits of the assay are 5.17, 5.09, and 45.4 ng/ml for WR 6026, WR 211,789 and WR 254,421 free base, respectively, with a signal to noise ratio of 3 to 1. Average mean recoveries over the working range of the standard curve were 97.2, 92.8, and 94.2 percent for WR 6026, WR 211,789 and WR 254,421 free base, respectively. The respective percent coefficients of variation (CVs) of the inter- and intraday assay precision analysis for the free base concentrations of WR 6026 ranged from 3.90% to 7.42% and 3.83% to 28.4%; of WR 211,789 ranged from 4.07% to 10.0% and 5.12% to 23.3%; and of WR 254,421 ranged from 3.09% to 5.86% and 3.55% to 10.0%. No discernible pattern of degradation was observed in long term or autosampler stability tests.

Study Report 24: Gentamicin and Paromomycin in Human and Rat Plasma

Study Characteristics: Study Report 24

Test Article:	Gentamicin Paromomycin
Test System:	human plasma rat plasma
Internal Standard:	Sisomicin
Sample Assay Volume:	0.2 ml
Sample Cleanup:	methyl *t*-butyl ether extraction

Analytical System: HPLC

Detector:	UV at 350 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5μ particle size
Mobile Phase:	16% CH_3CN, 0.2 M Na_2SO_4, 0.02 M sodium octanesulfonate, 0.1% acetic acid

Validation Results: Gentamicin free base in human plasma

Lower Limit of Quantitation:	0.100 μg/ml
Interday Mean, CV and RE:	7.88% and 7.00%
Intraday Mean, CV and RE:	15.7% and -14.9%
Standard curve range:	0.100-12.0 μg/ml
Interday Precision Concentrations:	0.200, 0.800, 2.50, and 5.00 μg/ml
CV Range:	4.34-8.42%
RE Range:	-3.20 to -0.800%
Intraday Precision Concentrations:	0.200, 0.800, 2.50, and 5.00 μg/ml
CV Range:	2.30-3.40%
RE Range:	-7.00 to -1.00%
Blind Sample Assay	
Concentration Range:	0.200-8.00 μg/ml
Bias Range:	-25.9 to +0.5%
Mean Recovery:	93.4%
Stability	
Plasma Freezer Storage:	-70°C for 12 months -20°C for 1 month
Processed Sample:	Room temp. for 3 days
Plasma Storage:	Room temp. for 6 hours
5 Cycle Freeze/Thaw:	5 cycles to -70°C
Standard Solution:	6 months

Validation Results: Paromomycin free base in human plasma

Lower Limit of Quantitation:	0.100 µg/ml
Interday Mean, CV and RE:	0.0960 µg/ml, 11.3% and -4.00%
Intraday Mean, CV and RE:	0.0867 µg/ml, 9.92% and -13.3%
Standard curve range:	0.100 to 12.0 µg/ml
Interday Precision Concentrations:	0.200, 0.800, 2.50, and 5.00 µg/ml
CV Range:	5.86 to 9.00%
RE Range:	-4.50 to +2.00%
Intraday Precision Concentrations:	0.200, 0.800, 2.50, and 5.00 µg/ml
CV Range:	3.12 to 3.96%
RE Range:	-7.50 to +1.87%
Blind Sample Assay	
Concentration Range:	0.200-9.00 µg/ml
RE Range:	-3.27 to +2.5%
Overall Mean Recovery:	93.8%
Stability	
Plasma Freezer Storage:	-70°C for 12 months
	-20°C for 1 month
Processed Sample:	Room temp. for 3 days
Plasma Storage:	Room temp. for 6 hours
5 Cycle Freeze/Thaw:	5 cycles to -70°C
Standard Solution:	6 months

Study Description: Gentamicin and Paromomycin in Human Plasma (the methodology was presented in DAMD17-92-C-2028 final report)

This report describes a high performance liquid chromatographic (HPLC) assay and provides data validating the assay for the aminoglycosides gentamicin and paromomycin. These compounds are used in a topical preparation for the treatment of cutaneous leishmaniasis.

Assays for gentamicin described in the literature include HPLC/MS/MS in bovine kidney,[42] fluorescent polarization immunoassay (FPIA) in dog and rabbit tears,[43] and a dipstick dot-ELISA in dairy milk.[44] Also, recently described is a comparative evaluation of FPIA and an automated homogeneous latex agglutination immunoassay for gentamicin.[45] Recent antimicrobial assays for paromomycin have been described for anticryptosporidial activity[46] and respiratory burst response of *Leishmania major* infected murine macrophages.[47] Standard anti-leishmanial drugs may be tested for ability to inhibit growth of intracellular amastigotes of *L. aethiopica*, *L. donovani* and *L. infantum* with use of the human leukemia monocyte cell line, THP-1.[48]

This report presents validation data for a method that employs an aqueous mobile phase, a C18 column, post column derivitization and fluorescence detection for concentration determinations in 0.2 ml human plasma samples with lower limits of quantitation of 0.100 µg/ml for both paromomycin and gentamicin.

Plasma samples were analyzed for gentamicin and paromomycin with an HPLC procedure that uses a C18 bonded column, an (acetonitrile/water) aqueous mobile phase, a *o*-pthaldialdehyde and 2 mercaptoethanol post column reagent, a fluorescence detector, and a 0.2 ml method sample size. Sample cleanup consisted of centrifugation. The methodology contains detailed procedures, which are summarized below.

Assay samples were prepared by spiking known volumes of human plasma with a known amount (constant over all samples in a run) of sisomicin internal standard (IS). Standard curve samples were generated by spiking interference free human plasma samples with known amounts of gentamicin and paromomycin (as free bases) and IS. These standard curve and assay samples were prepared for analysis, then 10 µl aliquots were injected onto the HPLC column for chromatographic separation and subsequent fluorometric detection of drug and IS peaks. The peak height ratios of gentamicin (free base) to IS and paromomycin (free base) to IS were calculated for each sample from the measured peak heights obtained by HPLC. Next, standard curve concentrations and gentamicin (free base) to IS or paromomycin (free base) to IS peak height ratios of the standard curve samples were fit by weighted least squares linear regression to the equation for the best straight line, $y = mx + b$, where y = peak height ratio and x = drug (free base) concentrations. Finally, drug concentrations in assay samples were calculated for each run by this equation from the gentamicin (free base) to IS or paromomycin (free base) to IS peak height ratios obtained by HPLC.

Plasma samples for analysis were thawed and mixed by vortexing (if appropriate), then pipetted (0.2 ml) into glass culture tubes. A constant amount (440 ng sisomicin in 20 µl of mobile phase) of IS and 20 µl of perchloric acid are added. Upon centrifugation, the resulting supernatant was transferred to a WISP vial and injected onto the column.

The assay described in the report dated January, 7, 1997, requires 0.2 ml plasma samples to determine the concentrations of gentamicin and paromomycin. The method involves centrifugation of plasma with injection of supernatant, separation on a C18 column with an aqueous mobile phase in an isocratic elution, a post column reaction and fluorescence detection. The advantages of this method include a clean baseline, a short run time, small sample size and a very simple sample preparation procedure.

The reversed-phase system (alkyl bonded silica gel with an aqueous mobile phase) is the most widely used HPLC technique in assays for drugs in biological fluids. In this kind of a system, the retention mechanism depends mainly on the lipophilic character of substances to be analyzed. Such a mechanism also retains considerable amounts of other lipophilic substances, thereby limiting the LLOQ to 100 µg/ml.

By use of the superior capability of fluorescence detection, the concentrations of gentamicin and paromomycin free base can be quantitatively and reliably measured in human and rat plasma samples. The drugs and IS are baseline

separated, and no interfering peaks were observed. The assay was demonstrated to be linear within the concentration ranges of the standard curves, 0.100 to 12 μg/ml for gentamicin and paromomycin as free bases. The CV (and corresponding RE) results of human plasma precision validation for gentamicin ranged from 4.34 to 8.42% (-3.20 to 0.800%, n=12) interday and 2.30 to 3.40% (-7.00 to -1.00%, n=6) intraday and for paromomycin ranged from 5.86 to 9.00% (-4.50 to +2.00%, n=12) interday and 3.12 to 3.96% (-7.50 to +1.87%, n=6) intraday. Mean calculated human plasma concentration results of replicate analyses (n=6) of samples spiked at the LLOQ (100 μg/ml for gentamicin and paromomycin) were 0.107 μg/ml (7.88% CV and +7.00% RE) interday and 0.0851 μg/ml (15.7% CV and -14.9% RE) intraday samples for gentamicin and 0.0960 μg/ml (11.3% CV and -4.00% RE) interday and 0.0867 μg/ml (9.92% CV and -13.3% RE) intraday samples for paromomycin. The signal to noise ratio was better than 2 to 1 for these LLOQ samples. The average recoveries from human plasma were 94.0% for gentamicin and 93.8% for paromomycin for the four concentrations within the standard curve quantitation limits. Stability test results indicate gentamicin and paromomycin are sufficiently stable in 1) prepared human plasma samples to withstand room temperature storage for at least 3 days, 2) human plasma at -70°C to permit storage without significant degradation for up to 1 year and at -20°C to permit storage without significant degradation for no longer than 1 month, 3) human plasma to withstand room temperature storage for at least 6 hours without significant degradation, and 4) human plasma to withstand 5 cycles of repeated freezing (to -70°C) and thawing without significant degradation. In the analyses of replicate (n = 5) blind samples the CV (and corresponding RE) of the results for four concentrations within the standard curve quantitation limits for gentamicin ranged 3.35-8.35% (-25.9 to +0.5%) and for paromomycin ranged 2.97-8.16% (-3.27 to +2.50%).

Short Validation Results: Gentamicin free base in rat plasma

Lower Limit of Quantitation:	0.100 µg/ml
Interday Mean, CV and RE:	0.0992 µg/ml, 4.29% and -0.800%
Intraday Mean, CV and RE:	0.0940 µg/ml, 5.42% and -6.00%
Standard curve range:	0.100-12.0 µg/ml
Interday Precision Concentrations:	0.200, 0.800, 2.50, and 5.00 µg/ml
CV Range:	3.51 to 8.80%
RE Range:	-5.93 to +4.96%
Intraday Precision Concentrations:	0.200, 0.800, 2.50, and 5.00 µg/ml
CV Range:	1.92 to 4.63%
RE Range:	-9.27 to +2.83%
Overall Mean Recovery:	93.4%

Short Validation Results: Paromomycin free base in rat plasma

Lower Limit of Quantitation:	0.100 µg/ml
Interday Mean, CV and RE:	0.0924 µg/ml, 10.8% and -7.57%
Intraday Mean, CV and RE:	0.0864 µg/ml, 8.08% and -13.7%
Standard curve range:	0.100-12.0 µg/ml
Interday Precision Concentrations:	0.200, 0.800, 2.50, and 5.00 µg/ml
CV Range:	6.30 to 12.4%
RE Range:	-4.90 to +10.6%
Intraday Precision Concentrations:	0.200, 0.800, 2.50, and 5.00 µg/ml
CV Range:	8.64 to 15.4%
RE Range:	-9.07 to +6.52%
Overall Mean Recovery:	68.2%

Study Description: Gentamicin and Paromomycin in Rat Plasma (the methodology was presented in DAMD17-92-C-2028 final report)

The CV (and corresponding RE, for n=6) results of rat plasma precision validation for gentamicin ranged from 3.51 to 8.80% (-5.93 to +4.96%) interday and 1.92 to 4.63% (-9.27 to +2.83%) intraday and for paromomycin ranged from 6.30 to 12.4% (-4.90 to +10.6%) interday and 8.64 to 15.4% (-9.07 to +6.52%) intraday. Mean calculated rat plasma concentration results of replicate analyses of samples spiked at the LLOQ (100 µg/ml for gentamicin and paromomycin) were 0.0992 µg/ml (4.29% CV and -0.800% RE, n=3) interday and 0.0940 µg/ml (5.42% CV and -6.00% RE, n=6) intraday samples for gentamicin and 0.0924 µg/ml (10.8% CV and -7.57% RE, n=3) interday and 0.0864 µg/ml (8.08% CV and -13.7% RE, n=6) intraday samples for paromomycin. The signal to noise ratio was better than 2 to 1 for these LLOQ samples. The average recoveries from rat plasma were 93.4% for gentamicin and 68.2% (which is noticeably lower than in human plasma) for paromomycin for the four concentrations within the standard curve quantitation limits.

Study Report 26: WR 242511 in Human and Dog Plasma

Study Characteristics: Study Report 26

Test Article:	WR 242511
Test System:	human plasma dog plasma
Internal Standard:	Chlorpheniramine maleate
Sample Assay Volume:	0.5 ml
Sample Cleanup:	methyl *t*-butyl ether extraction

Analytical System

Detector:	UV at 350 nm
Column Type:	silica
Column Size:	4.6x250 mm, 5µ particle size
Mobile Phase:	CH_3CN/H_2O (7:3, v/v) with 0.008% TEA and 0.005% H_3PO_4 (final concentrations)

Validation Results: WR 242511 free base in human plasma

Lower Limit of Quantitation:	4.00 ng/ml
Interday Mean, CV and RE:	4.57 ng/ml, 5.84% and 14.2%
Intraday Mean, CV and RE:	3.69 ng/ml, 7.87% and -15.6%
Standard curve range:	4.00 to 1024 ng/ml
Interday Precision Concentrations:	8.00, 32.0, 128, and 256 ng/ml
CV Range:	8.74 to 11.9%
RE Range:	-3.32 to +5.40%
Intraday Precision Concentrations:	8.00, 32.0, 128, and 256 ng/ml
CV Range:	2.99 to 5.90%
RE Range:	+5.21 to +12.3%
Blind Sample Assay	
Concentration Range:	4.70 to 822 ng/ml
RE Range:	-6.27 to +21.9%
Overall Mean Recovery:	77.1%
Stability	
Plasma Freezer Storage:	-70°C for 6 months -20°C for 6 months
Processed Sample:	Room temp. for 4 days
Plasma Storage:	Room temp. for 6 hours
5 Cycle Freeze/Thaw:	5 cycles to -70°C
Standard Solution:	6 months

Short Validation Results: WR 242511 free base in dog plasma

Lower Limit of Quantitation:	4.00 ng/ml
Precision Mean, CV and RE:	4.42 ng/ml, 6.52% and +10.4%
Standard curve range:	4.00 to 1024 ng/ml
Interday Precision Concentrations:	8.00, 32.0, 128, and 256 ng/ml
CV Range:	4.14 to 13.3%
RE Range:	-5.78 to -0.104%
Intraday Precision Concentrations:	8.00, 32.0, 128, and 256 ng/ml
CV Range:	0.764 to 5.12%
RE Range:	-10.8 to -3.35%
Overall Mean Recovery:	79.3%

Study Description: WR 242511 in Human and Dog Plasma (the methodology was presented in DAMD17-92-C-2028 final report)

This report describes a high performance liquid chromatographic (HPLC) assay and provides data validating the assay for a compound of the 8-aminoquinoline class. The compound, 8-[(4-amino-1-methylbutyl)amino]-5-(1-hexyloxy)-6-methoxy-4-methylquinoline (DL) tartrate (WR 242511, Figure 1), holds promise[49] in an effort to replace primaquine, the radical cure and prophylaxis for vivax and ovale malaria and is being developed by WRAIR as an anti-cyanide drug.

Assays for other 8-aminoquinolines include high performance liquid chromatography (HPLC) methods with electrochemical,[50] ultraviolet,[51] and fluorescence[52] detection. An HPLC method with oxidative electrochemical detection has been described[53] for WR 242511 in 0.25 ml plasma samples with a detection limit of 10 ng/ml. This report presents validation data for a superior method that employs an aqueous mobile phase, an unbonded silica gel column[54] and ultraviolet detection for WR 242511 free base concentration determinations in 0.5 ml human and dog plasma samples with a lower limit of quantitation of 4 ng/ml.

Plasma samples were analyzed for WR 242511 free base with an HPLC procedure that uses a silica gel column, an (acetonitrile/water) aqueous mobile phase, UV absorbance detection, and a 0.5 ml method sample size. Sample cleanup consisted of extraction into methyl *t*-butyl ether. The methodology contains detailed procedures, which are summarized below.

Assay samples were prepared by spiking known volumes of human plasma with a known amount (constant over all samples in a run) of CPA internal standard (IS). Standard curve samples were generated by spiking a known amount of WR 242511 tartrate into interference free human plasma which is then brought to a known volume, divided by serial dilution and spiked with a known amount of IS. These standard curve and assay samples were prepared for analysis, then 40 µl aliquots were injected onto the HPLC column for

chromatographic separation and subsequent UV absorbance detection of drug and IS peaks. The peak height ratios of WR 242511 to IS were calculated for each sample from the measured peak heights obtained by HPLC. Next, standard curve concentrations and WR 242511 to IS peak height ratios of the standard curve samples were fit by 1/y weighted least squares linear regression to the equation for the best straight line, $y = mx + b$, where y = peak height ratio and x = WR 242511 free base concentrations. Finally, drug concentrations in assay samples were calculated for each run by this equation from the WR 242511 to IS peak height ratios obtained by HPLC.

Stock solutions of WR 242511 tartrate and chlorpheniramine maleate internal standard (IS) were stored in a 4°C refrigerator and protected against exposure to light as necessary, and checked for deterioration by following the ratio of drug to internal standard peak heights in a diluted solution (solutions are discarded when a more than 10% change in the ratio is observed or by 2 months after the preparation date).

Plasma samples for analysis were thawed and mixed by vortexing (if appropriate), then pipetted (0.5 ml) into glass culture tubes. A constant amount (1.0 µg chlorpheniramine maleate) of IS, 100 µl of 0.1N NaOH, and 3 ml of methyl *t*-butyl ether are added. Upon centrifugation and freezing of the aqueous layer, the resulting supernatant was transferred to a clean tube, evaporated to dryness, reconstituted in 70% acetonitrile, transferred to WISP vials, and injected onto the column.

The assay described in the report dated December, 12, 1996, requires 0.5 ml plasma samples to determine the concentrations of WR 242511 free base. The method involves extraction from plasma with methyl *t*-butyl ether, separation on a silica gel column with an aqueous mobile phase in an isocratic elution, and ultraviolet absorbance detection. The advantages of this method include a clean baseline and a short run time.

The reversed-phase system (alkyl bonded silica gel with an aqueous mobile phase) is the most widely used HPLC technique in assays for drugs in biological fluids. In this kind of a system, the retention mechanism depends mainly on the lipophilic character of substances to be analyzed. Such a mechanism also retains considerable amounts of other lipophilic substances, thereby interfering with the drug peak. On the other hand, in a system consisting of a bare silica gel and an aqueous mobile phase, the retention mechanism results mainly from ion exchange[6] and only partially from lipophilic interactions. Thus, endogenous non-ionic neutral lipid compounds and anionic compounds will not be retained on the silica gel column; only the cationic (e.g. ammonium) ions will be retained. The interfering substances in biological fluids elute at the solvent front, leaving a very clean baseline around the retention time of the drug. In this method, the mobile phase is recycled through a non analytical silica gel column overnight to saturate it with silica. Overnight saturation of mobile phase prior to use is beneficial to the whole system, since silica gel slowly dissolves in neutral aqueous solution and the water flowing through the silica gel approaches the equilibrium concentration of silica. The saturated mobile phase does not

dissolve silica from the analytical columns and degradation of the analytical column is decreased relative to the single pass system.

By use of a solvent extraction step for sample clean-up, an unbonded silica gel column combined with an aqueous mobile phase for separation, and the superior capability of ultraviolet detection, the concentration of WR 242511 free base can be quantitatively and reliably measured in human and dog plasma samples. The drug and IS are baseline separated, and no interfering peaks were observed. The assay was demonstrated to be linear within the range of the standard curve, 4.00 to 1024 ng/ml WR 242511 free base. The CVs of results for human plasma precision validation ranged from 8.74 to 11.9% interday and 2.99 to 5.90% intraday, while percent RE of measured results compared to serially diluted concentrations ranged -3.32 to +5.40% interday and +5.21 to +12.3% intraday for WR 242511 free base. The mean concentrations (n = 6) obtained for human plasma samples serially diluted to the LLOQ (4.00 ng/ml) were 4.57 ng/ml interday (5.84% CV and +14.2% RE) and 3.38 ng/ml intraday (7.87% CV and -15.6% RE) where the signal to noise ratio was better than 3 to 1. WR 242511 average recovery from human plasma extraction for the four concentrations within the standard curve quantitation limits was 77.1%. Stability test results indicate WR 242511 is sufficiently stable in 1) human plasma samples prepared for assay (includes extraction, evaporation, and reconstitution in 70% acetonitrile) to withstand room temperature (RT) storage for at least 4 days, 2) human plasma at -70°C and at -20°C to permit storage without significant degradation for up to 6 months, 3) human plasma to withstand RT storage for at least 6 hours without significant degradation, and 4) human plasma to withstand 5 cycles of repeated freezing in a -70°C freezer and thawing at room temperature without significant degradation. The CVs of the results for the analyses of blind WR 242511 human plasma samples (n = 5) at five concentrations within the standard curve limits ranged 0.958-9.61% while R.E.s ranged -8.39 to +21.9%.

The CV (and corresponding RE, for n = 6) results of dog plasma precision validation for WR 242511 ranged from 4.14 to 13.3% (-5.78 to -0.104%) interday and 0.764 to 5.12% (-10.8 to -3.35%) intraday. Mean back calculated dog plasma concentration results of replicate analyses of precision standard curve samples serially diluted to the LLOQ (4.00 ng/ml for WR 242511 free base concentration) was 4.42 ng/ml (6.52% CV and +10.4% RE, n = 4). The signal to noise ratio was better than 3 to 1 for these LLOQ samples. WR 242511 average recovery from dog plasma extraction for the four concentrations within the standard curve quantitation limits was 79.3%.

Study Report 27: WR 238,608 R and S Isomers in Human Plasma

Study Characteristics: Study Report 27

Test Article:	WR 238,608 (R isomer) WR 238,608 (S isomer)
Test System:	human plasma
Internal Standard:	WR 211,789
Sample Assay Volume:	1.00 ml
Sample Cleanup:	methyl *t*-butyl ether double extraction

Analytical System

Detector:	fluorescence-Ex: 375 nm; Em: 480 nm
Column Type:	Chiralcel OD-R, cellulose tris
Column Size:	4.6x250 mm, 10µ particle size
Mobile Phase:	acetonitrile/$NaClO_4$ (45:55, v/v) at pH 5.
Standard curve range:	5.00 to 1000 ng/ml

Validation Results: *S* (+) WR 238,608 free base in human plasma

Lower Limit of Quantitation:	5.00 ng/ml
Interday Mean, CV and RE:	5.50 ng/ml, 10.5% and +10.1%
Intraday Mean, CV and RE:	5.50 ng/ml, 3.93% and +9.93%
Standard curve range:	5.00 to 1000 ng/ml
Interday Precision Concentrations:	10, 50, 200, and 800 ng/ml
CV Range:	5.71 to 12.7%
RE Range:	-5.13 to +230 %
Intraday Precision Concentrations:	10, 50, 200, and 800 ng/ml
CV Range:	5.89 to 14.1 %
RE Range:	-0.6 to +5.58%
Blind Sample Assay	
Concentration Range:	Not determined
RE Range:	Not determined
Overall Mean Recovery:	109%
Stability	
Plasma Freezer Storage:	Not determined
Processed Sample:	Not determined
Plasma Storage:	Not determined
5 Cycle Freeze/Thaw:	Not determined
Standard Solution:	Not determined

Study Description: WR 238,608 R and S Isomers in Human Plasma (the methodology is presented in Appendix A)

This project was requested as described in a COR letter dated Oct. 20, 1995. Standards of the enantiomers requested Oct. 4, 1996 were received on October 24, 1996.

This report describes a high performance liquid chromatographic (HPLC) assay and provides data validating the assay for the R and S isomers of WR 238605. The compound, WR 238,605 (free base) ((8-[(amino-1-methylbutyl)amino]-2,6-dimethoxy-4-methyl-5-(3-trifluoromethylphenoxy) quinoline succinate), an 8-aminoquinoline derivative (Figure 1), is the Walter Reed Army Institute of Research (WRAIR) drug development program's leading drug candidate in an effort to find safer and more effective drugs to deal with chloroquine-resistant falciparum malaria and the severe life-threatening side effects of and resistance to combination drug treatments.[55]

Assays for other 8-aminoquinolines include high performance liquid chromatography (HPLC) methods with electrochemical,[56] ultraviolet,[57] and fluorescence[58] detection. A chiral method for the determination of WR 238605 free base concentrations has been reported.[59] This report presents validation data for a method that employs an aqueous mobile phase, a chiral cellulose tris column and fluorescence detection for the R and S enantiomers of WR 238605 free base concentration determinations in 1 ml human plasma samples with a lower limit of quantitation of 5 ng/ml.

Plasma samples were analyzed for the R and S enantiomers of WR 238605 free base with an HPLC procedure that uses a chiral cellulose tris column, an (acetonitrile/water) aqueous mobile phase, fluorescence detection, and a 1 ml method sample size. Sample cleanup consisted of extraction into methyl *t*-butyl ether. The methodology contains detailed procedures, which are summarized below.

Assay samples were prepared by spiking known volumes of human plasma with a known amount (constant over all samples in a run) of WR 211789 internal standard (IS). Standard curve samples were generated by spiking interference free human plasma samples with known amounts of WR 238605 (free base) and IS. These standard curve and assay samples were prepared for analysis, then 10 to 100 µl aliquots were injected onto the HPLC column for chromatographic separation and subsequent fluorescence detection of drug and IS peaks. The peak height ratios of WR 238605 (free base) R and S isomers to IS were calculated for each sample from the measured peak heights obtained by HPLC. Next, standard curve concentrations and R or S isomer WR 238605 (free base) to IS peak height ratios of the standard curve samples were fit by weighted least squares linear regression to the equation for the best straight line, $y = mx + b$, where y = peak height ratio and x = R or S isomer WR 238605 (free base) concentrations. Finally, drug concentrations in assay samples were calculated for each run by this equation from the R or S isomer WR 238605 (free base) to IS peak height ratios obtained by HPLC.

Stock solutions of WR 238605 succinate and WR 211789 dihydrochloride internal standard (IS) were stored in a 4°C refrigerator and protected against exposure to light as necessary, and checked for deterioration by following the ratio of drug to internal standard peak heights in a diluted solution (solutions are discarded when a more than 10% change in the ratio is observed or by 6 months after the preparation date).

Plasma samples for analysis were thawed and mixed by vortexing (if appropriate), then pipetted (1.0 ml) into glass culture tubes. A constant amount (6.49 µg of WR 211789 - in 75 µl of 86.5 µg/ml 50% methanol solution) of IS, 100 µl of 1M NaOH, and 3 ml of methyl *t*-butyl ether are added. Upon centrifugation and freezing of the aqueous layer, the resulting supernatant was transferred to a clean tube. A second 3 ml methyl *t*-butyl ether extraction of the plasma was performed, and the combined supernatants were evaporated to dryness, reconstituted in 200 µl of mobile phase, transferred to WISP vials, and injected onto the column.

The assay described in this report requires 1.00 ml plasma samples for determinations of the R and S isomer concentrations of WR 238605 free base. The method involves extraction from plasma with methyl *t*-butyl ether, separation on a cellulose tris column with an aqueous mobile phase in an isocratic elution, and fluorescence detection.

By use of a solvent extraction step for sample clean-up, a chiral column in a reverse phase system for separation, and the superior capability of fluorescence detection, the concentration of the enantiomers of WR 238605 free base can be quantitatively and reliably measured in human plasma samples. Sample throughput is estimated at 10 hours for one person to prepare approximately 43 samples (including standard curve and control samples) for a 38 hour run. The drug and IS are baseline separated, and no interfering peaks were observed. The assay was demonstrated to be linear within the range of the standard curves, 5.00 to 1000 ng/ml for the R and S isomers of WR 238605 free base. The assay was demonstrated to be precise and accurate within the limits of the standard curve range and at the LLOQ by acceptable CV and RE values in replicate spiked sample analyses. Acceptable extraction recovery from human plasma was demonstrated. Stability tests were not performed; stability data was obtained in Study Report 13B,[4] which indicated plasma samples remain stable in plasma at -20°C up to 4 months. Additional stability tests are underway and will be presented in Study Report 32.

Study Report 28: Halofantrine and WR 178460 R&S Isomers in Human Plasma

Study Characteristics: Study Report 28

Test Article:	Halofantrine (R isomer) Halofantrine (S isomer) WR 178460 (R isomer) WR 178460(S isomer)
Test System:	human plasma

This project was requested as described in a COR letter dated Oct. 20, 1995. WR 216062 and WR 216063 standard samples were received October 20, 1995 for use in development and validation of an assay for halofantrine and desbutylhalofantrine enantiomers in human plasma. A draft report is in preparation.

Study Report 29: Chloroquine and Metabolites in Human Blood

Study Characteristics: Study Report 29

Test Article:	Chloroquine Monodesethyl Chloroquine Didesethyl Chloroquine
Test System:	human blood
Internal Standard:	Neostigmine bromide
Sample Assay Volume:	100 µl
Sample Cleanup:	Lyse cells with water precipitate proteins with acetonitrile

Analytical System

Detector:	MS/MS
Column Type:	Hypersil silica
Column Size:	4.6x50 mm, 3 µ particle size
Mobile Phase:	CH_3CN/H_2O (9:1, v/v) with TFA and 5mM ammonium acetate

Validation Results: chloroquine free base in human blood

Lower Limit of Quantitation:	20 ng/ml
Interday Mean, CV and RE:	16.9 ng/ml, 5.93% and –15.4%
Intraday Mean, CV and RE:	22.6 ng/ml, 9.81% and +12.9%
Standard curve range:	20 to 2000 ng/ml
Interday Precision Concentrations:	40, 100, 250, and 1500 ng/ml
CV Range:	4.57 to 11.4%
RE Range:	-7.49 to –1.99 %
Intraday Precision Concentrations:	40, 100, 250, and 1500 ng/ml
CV Range:	2.04 to 8.22 %
RE Range:	-8.08 to +0.210%
Blind Sample Assay	
Concentration Range:	Not determined
RE Range:	Not determined
Overall Mean Recovery:	70.4%
Stability	
Plasma Freezer Storage:	Not determined
Processed Sample:	4°C for 27 days
Plasma Storage:	Room temp. for 6 hours
5 Cycle Freeze/Thaw:	5 cycles to -20°C
Standard Solution:	Not determined

Study Description: Chloroquine and Metabolites in Human Plasma (the methodology is presented in Appendix A)

This highest priority project was requested in a COR letter dated December 6, 1995. Additional standard compounds were received for chloroquine diphosphate (WR 1544), the didesethyl chloroquine metabolite (WR 112472) and the monodesethyl chloroquine metabolite (WR 29623) on October 24, 1996.

This report describes the analytical method and validation of the analytical method used to measure concentrations of chloroquine and monodesethyl-chloroquine in human blood samples. The method was developed and validated at the Analytical Division, Drug Studies Unit (DSU), UCSF, San Francisco, CA. The analysis of human blood samples was accomplished by use of the liquid chromatographic/mass spectrometric/mass spectrometric (LC/MS/MS) method.

Method Summary: Human blood samples (100 µl) were analyzed for chloroquine free base (CHL), and monodesethyl-chloroquine (MDC) with an LC/MS/MS procedure in a PE Sciex-API III® system that uses a silica gel column, an acetonitrile/water/TFA (90:10:0.1, v/v) with 5 mM ammonium acetate mobile phase, and mass spectrometric detection with sample inlet by heated nebulizer, positive ionization by APCI (Atmospheric Pressure Chemical Ionization) and mass scanning by MRM (Multiple Reaction Monitoring) analysis. Sample cleanup consisted of addition of water and sonication to lyse cells, precipitation with a neostigmine internal standard solution in acetonitrile, centrifugation and transfer of the supernatant prior to separation by LC/MS/MS.

Validation Results Summary: The following validation parameters were evaluated for CHL and MDC.

1. Specificity: No significant endogenous interfering peaks for CHL, MDC, or for the internal standard were observed in blank human blood.

2. Inter-Day Precision and Accuracy: Interday precision and accuracy measurements were determined by analyzing quality control (QC) samples made of human blood spiked with known amounts of drug and metabolite. Each of 6 sets (n=2) of control samples at 4 different drug and metabolite concentrations was evaluated (6 standard curves for the drug and metabolite were run). Precision coefficients of variation (CV), ranged from 7.57% to 11.4% for CHL and from 8.51% to 13.0% for MDC. The accuracy, defined by the relative error (RE) ranged from -7.49% to -1.99% for CHL and from -8.28% to -4.87% for MDC.

3. Intra-Day Precision and Accuracy: Intraday precision and accuracy measurements were determined by analyzing quality control (QC) samples made of human blood spiked with known amounts of drug and metabolite. For intraday precision, 6 sets (n=1) of control samples for each

of 4 different drug and metabolite concentrations were evaluated with 1 standard curve on the same run. CVs ranged from 2.04% to 8.22% for CHL and from 2.62% to 9.84% for MDC. R.E.s ranged from -8.08% to +0.210% for CHL and from -11.4% to +0.103% for MDC.

4. Lower Limit of Quantitation (LLOQ): The LLOQs for this assay are equivalent to the low points of the standard curves, or 20.0 ng/ml each for CHL and MDC. Interday mean, CV, and RE results are 16.9 ng/ml, 5.93%, and -15.4% for CHL and 17.8 ng/ml, 11.0%, and -10.8 for MDC. Intraday mean, CV, and RE results are 22.6 ng/ml, 9.81%, and +12.9% for CHL and 22.2 ng/ml, 8.34%, and +11.2% for MDC.

5. Linear Range: The validated linear concentration ranges for this assay were 20.0 to 2000 ng/ml for CHL and MDC.

6. Recovery: Peak area ratios of processed samples to unprocessed samples provided overall recoveries of 70.4% for CHL and 61.3% for MDC.

7. Stability:

   a. Freeze/Thaw: CHL and MDC were shown to be stable in human blood for up to 5 freeze/thaw cycles when samples are frozen to -20°C and thawed to room temperature.

   b. Bench Top: CHL and MDC were shown to be stable for at least 6 hours in human blood at ambient temperature.

   c. Processed Samples: CHL, MDC, and internal standard were shown to be stable up to 27 days at 4°C. A test at the ambient temperature is yet to be performed.

   d. Long Term: A test of stability of -20°C freezer storage is yet to be performed.

Human blood samples (100 µl) were analyzed for chloroquine and monodesethyl-chloroquine with an LC/MS/MS procedure in a PE Sciex-API III system equipped with a silica column (4.6 x 50 mm, 5 µm particle size), 90% CH_3CN, 0.1% trifluoroacetic acid (TFA) and (final concentration) 5 mM ammonium acetate mobile phase and mass spectrometric detection with sample inlet by heated nebulizer, positive ionization by APCI (atmospheric pressure chemical ionization) and mass scanning by MRM (Multiple Reaction Monitoring) analysis. Sample preparation consisted of addition of 50 µl of water, sonication of the mixture, addition of neostigmine bromide internal standard (IS), and transfer of the supernatant prior to separation by LC/MS/MS. Standard curve and quality control (QC) samples were generated by spiking interference free human blood samples with known amounts of chloroquine, monodesethyl-chloroquine, and IS. Standard curve, QC and assay samples were prepared as described, then 1-2 µl aliquots were injected into the LC/MS/MS system for chromatographic separation and subsequent mass spectrometric detection. The

peak area ratios of chloroquine and monodesethyl-chloroquine to IS were calculated for each sample from the measured peak areas obtained by LC/MS/MS. Finally, spiked concentrations and chloroquine and monodesethyl-chloroquine to IS peak area ratios of the standard curve samples were fit by 1/y weighted least squares linear regression to the two equations for the best straight lines ($y = mx + b$, where y = peak area ratio and x = chloroquine or monodesethyl-chloroquine concentrations), and drug and metabolite concentrations in assay samples were calculated by these equations from the chloroquine and monodesethyl-chloroquine to IS peak area ratios obtained by LC/MS/MS.

Calibration standards and validation samples with drug concentrations within the calibration range of the assay were analyzed to assess the performance of the assay. Calibration standards and validation samples were generated by spiking blank human blood with chloroquine and monodesethyl-chloroquine. A didesethyl-chloroquine stock solution was also generated, diluted into working solutions and spiked into validation samples but data is not presented in this report, since validation acceptance criteria were not met.

Conclusion: The LC/MS/MS method for analysis of human blood to determine concentrations of CHL and MDC was validated for the concentration ranges of 20.0 ng/ml to 2000 ng/ml for CHL and MDC. The method was demonstrated to be precise, accurate, and sufficiently reproducible for analysis of study samples.

Study Report 30: WR 243251 in Human Plasma

Study Characteristics: Study Report 30	
Test Article:	WR 243251
Test System:	human plasma
Analytical System	
Detector:	MS/MS

This project was requested in a COR letter dated May 6, 1996. WR 243,251 standard compound was received May 10, 1996. Method development is in progress.

Study Report 31: WR 238,608, Mefloquine, Chloroquine, Quinine, and Doxycycline in Dog Plasma

Study Characteristics: Study Report 31

Test Article:	WR 238,608 Halofantrine Mefloquine Chloroquine Quinine Doxycycline
Test System:	dog plasma
Analytical System	
Detector:	MS/MS

A study on WR 238,605 used in combination with mefloquine, chloroquine, halofantrine, quinine and doxycycline in dog plasma as described in a COR letter dated Oct. 20, 1995 continued. A draft report is in preparation.

Study Report 32: WR 238,608 in Human Plasma

Study Characteristics: Study Report 32

Test Article:	WR 238,608
Test System:	human plasma
Internal Standard:	Verapamil
Sample Assay Volume:	100 µl
Sample Cleanup:	acetonitrile precipitation
Analytical System	
Detector:	MS/MS
Column Type:	hypersil silica
Column Size:	4.6x50 mm, 5µ particle size
Mobile Phase:	acetonitrile/water/TFA (90:10:0.06, v/v/v).

Validation of a method for WR 238,605 in human plasma and blood by LC/MS/MS has been completed and a report is in preparation. A long term stability study for WR 238,605 in human blood and plasma (plasma data to 21 months and blood to 7 months was Faxed to the COR on May 22, 1998) for up to two years requested in a COR letter dated Feb. 15, 1996 continued. Results on analyses of 35 blind human plasma samples received February 25, 1998 from WRAIR were Faxed to the COR on May 21, 1998.

A small volume sample (50 µl blood) method described in Faxes from the COR dated April 13, 21 and 24, 1998 has been validated and a report is in preparation.

Study Report 33: Halofantrine and WR 178460 in Human Plasma and Blood

Study Characteristics: Study Report 33

Test Article:	Halofantrine WR 178460
Test System:	human plasma and blood
Analytical System	
Detector:	MS/MS

Long term (2 year) freezer stability study and LC/MS/MS method development is in progress for halofantrine and desbutylhalofantrine in human plasma and blood.

Study Report 34: WR 254421 in Human Plasma

Study Characteristics: Study Report 34

Test Article:	WR 254421
Test System:	human plasma
Analytical System	
Detector:	MS/MS

This project was requested as described in a COR fax dated November 12, 1997 for quantitation of WR 254,421 (as Free Base) in human plasma. Assay development is in progress.

Study Report 35: Artelinic Acid in Rat Plasma

Study Characteristics: Study Report 35	
Test Article:	Artelinic acid
Test System:	rat plasma
Analytical System	
Detector:	MS/MS

LC/MS/MS method development is in progress for WR 254421 in human plasma. This project was described in a COR fax dated July 22, 1998.

Routine Assay Results

The following section presents short descriptions of specific routine sample assays completed or currently in progress during the contract. Complete annual data findings are presented in Appendix B.

TABLE 5: CURRENT ROUTINE ANALYSES

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine analysis for Halofantrine and WR 178,460 (as f.b.) of Plasma Samples Obtained for the Protocol Titled "Pharmacokinetics of a New Multiple Dose Halofantrine Regimen"	12/10/96 in review	Halofantrine WR 178,460	Human plasma	642 642	Hal/P 93-2
No protocol	2/25/94 data, draft in preparation	*p*-aminoheptanophenone	dog plasma	876	Pah/P 93-3
Routine Analysis for WR 238,605 (as f.b.) of Plasma Samples Obtained for the Protocol Titled "Thirteen Week Oral Toxicity Study of WR 238,605 with a Thirteen Week Recovery Period in Dogs"	Draft 4/25/94 in revision	WR 238,605	dog plasma	330	WR5/P 93-4
Routine Analysis for WR 238,605 (as f.b.) of Plasma Samples Obtained for the Protocol Titled "Thirteen Week Oral Toxicity Study of WR 238,605 with a Thirteen Week Recovery Period in Rats"	11/4/96 amendment accepted as final 7/27/98	WR 238,605	rat plasma	154	WR5/P 93-5
Routine Analysis for Halofantrine and WR 178,460 (as f.b.) of Rat Liver, Bile and Perfusate Samples	10/28/94 final data, draft report in preparation	halofantrine	rat liver perfsate bile		Hal/lpb 93-7
Routine Analysis for WR 238,605 (as f.b.) Human Plasma and Blood Samples Obtained for the Protocol Titled "Pharmacokinetics, Pharmacodynamics, Safety and Tolerance of a Single Oral Dose of WR 238605 Succinate"	9/16/94 final data, draft report in preparation	WR 238,605	human plasma blood	359 359	WR5/PB 93-8

TABLE 5: CURRENT ROUTINE ANALYSES

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for *p*-Aminoheptanophenone of Dog Plasma Samples Obtained for the Protocol Titled "*p*-Amino-heptanophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Dogs"	2/7/95 final data, draft report in prepara-tion	*p*-aminohept-anophenone	dog plasma	189	Pah/P 93-9
Routine Analysis for WR 238,605 (as free base)Monkey Plasma Samples	11/22/94 final data, letter report in preparatio n	WR 238,605	monkey plasma	12	WR5/P 94-1
Routine Analysis for *p*-Amino-heptanophenone Rat Plasma Samples Obtained for the Protocol Titled "p-Aminoheptanophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Rats"	2/7/95 final data, draft in prepara-tion	*p*-aminohept-anophenone	rat plasma	152	Pah/P 94-2
Tentative Title: Routine Analysis for WR 6026 and Metabolites in Plasma and Urine Samples Obtained for the Protocol Titled "Clinical Trial of Oral WR6026•2HCl in Patients with Brazilian Visceral Leishmaniasis due to L. chagasi: Initial Dose Range Determine	8/22/97 final data final data in proges final data final data final data	 WR 6026 WR 211789 WR 254421 WR 6026 WR 211789 WR 254421	human plasma urine	 120 90	WR6/PU 94-3
Tentative Title: Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "Evaluation of WR 238605 as a Prophylactic Agent against Induced P. falciparum Malaria Infection in Healthy Non-immune Subjects: A Dose Ranging Study"	11/21/94 final data, draft report in preparatio n	WR 238,605	human plasma blood	 28 28	WR5/PB 94-4

TABLE 5: CURRENT ROUTINE ANALYSES

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Tentative Title: Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "A Multiple Dose Safety, Tolerance and Pharmacokinetic Study of WR 238605 when Given to Healthy Male and Female Subjects"	8/27/98 final report	WR 238605	human plasma	709	WR5/P 94-7
Tentative Title: Routine Analysis for WR 238605 in Rat Plasma Samples Obtained for the Protocol Titled "Six Month Oral Toxicity Study of WR 238605 Succinate in Rats	7/24/98 final report	WR 238605	rat plasma	405	WR5/P 95-1
Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "Evaluation of WR 238605 as a Prophylactic Agent Against Induced P. Falciparum Malaria Infection in Healthy Non-Immune Subjects II: A Multiple Dose Causal versus Suppressive	8/28/98 final report	WR 238605 chloroquine monodesethyl chloroquine	human plasma blood blood blood	226 226 67 67	WR5/P 95-2
Routine Analysis for R and S Isomers of WR 238605 of Human Plasma Samples Obtained for the Protocol Titled "Evaluation of WR 238605 as a Prophylactic Agent Against Induced P. Falciparum Malaria Infection in Healthy Non-Immune Subjects II: A Multiple Dose Causal versus Suppressive	9/8/98 Final report	*R* WR 238605 *S* WR 238605	human plasma	226 226	WR5/P 95-2
Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "WR 238605 Multiple Drug Interaction Study in Beagle Dogs"	8/26/98 prelimin-ary data	WR 238605 Mefloquine Chloroquine Quinine Doxycycline Halofantrine	human plasma	1084	WR5/P 95-3

TABLE 5: CURRENT ROUTINE ANALYSES

Report Title	Report Date	Test Article	Test System	No. of Samples	Report No.
Routine Analysis for Halofantrine and WR 178460 in Plasma Samples Obtained for the Protocol Titled "Halofantrine as Prophylaxis against Malaria: Multiple-Dose Safety, Tolerance and Pharmacokinetics Study"	11/24/97 final data chiral 5/4/98 final data Draft report in preparation	Halofantrine WR 178,460 Halofantrine WR 178,460	human plasma	1365? 1365? 1365? 1365?	Hal/P 95-4
Routine Analysis for Halofantrine and WR 178460 in Aotus Monkey Blood Samples	1/23/98 Final report	Halofantrine	monkey blood	165	Hal/B 96-1
Tentative Title: Routine Analysis for WR 238605 in Plasma Samples Obtained for the Protocol Titled "Dose-Ranging Study of the Safety and Efficacy of WR 238605 in the Prevention of Relapse of Plasmodium vivax Infection in Thailand"	3/2/98 final data submitted assay in progress	WR238605 Chloroquine	human plasma blood	558 552	WR5/BP 96-2
Tentative Title: Routine Analysis for Gentamicin and Paromomycin in Human Plasma Samples	3/2/98 draft report submitted	Gentamicin Paromomycin	Human plasma	36 47	Gnt/P 96-3
Routine Analysis for WR238605 in Dog Plasma Samples for the Protocol Titled "One Year Oral Toxicity Study of WR 238605"	9/26/97 final data submitted draft report in preparation	WR238605	Dog plasma	224	WR5/P 97-1
Routine Analysis for Mefloquine Chloroquine and Primaquine in Plasma Samples	12/11/97 final data, draft report in preparation	Mefloquine Chloroquine Primaquine	Plasma	14 2 2	MEF/P 97-2

p-Aminoheptanophenone (WR 269,410), WR 258,948 and WR 302

Pah/P 93-3
(analytical data was presented in the DAMD17-92-C-2028 mid-term report)

Results will be reported in Analysis Report No. 93-3. Status of samples received is described in the table below. Report completion requires completion of method validation.

No. of Samples	Description	Date Received	Status
106	dog plasma	3/3/93	Results Faxed to COR 9/23/93
52	dog blood	3/3/93	Not to be assayed
645	dog plasma	9/21/93	Results Faxed to COR 2/25/94
36	blind spiked dog plasma	9/30/93 11/2/93	Results Faxed to COR 1/25/94
125	dog plasma	10/21/93	Results Faxed to COR 2/25/94

Pah/P 93-9
(analytical data was presented in the DAMD17-92-C-2028 final report)

Samples (189 dog plasma) were received July 12, 1994 to be analyzed in accordance with the protocol titled "*p*-Aminoheptanophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Dogs." Analysis is complete and final results were Faxed to the COR on February 7, 1995. Report completion requires completion of the method validation report.

Pah/P 94-2
(analytical data was presented in the DAMD17-92-C-2028 final report)

Samples (152 rat plasma) were received July 12, 1994 to be analyzed in accordance with the protocol titled "*p*-Aminoheptanophenone (PAHP) (WR269410) Single Dose IV and Oral Pharmacokinetic, Pharmacodynamic, Bioavailability and Metabolism Study in Rats." Analysis is complete and final results were Faxed to the COR on February 7, 1995. Report completion requires completion of the method validation report.

HALOFANTRINE

Hal/P 93-2
(analytical data was presented in the DAMD17-92-C-2028 final report)

Analysis of 642 human plasma samples for determination of the free base concentrations of halofantrine (WR 171,669) and of its metabolite (WR 178,460) was accomplished by use of an HPLC method described in Study Report 17, developed under contract DAMD17-86-C-6150. The samples were obtained from the South Florida Drug Research Corporation, Inc., in accordance with the protocol titled "Pharmacokinetics of a New Multiple Dose Halofantrine

Regimen." Analytical results were presented in Analysis Report Hal/P 93-2 submitted for review on December 10, 1996, for plasma samples from human male subjects from analyses performed from April 30 through June 8, 1993.

Hal/lpb 93-7
(analytical data was presented in the DAMD17-92-C-2028 final report)

Final bile, liver and perfusate results were attached to Quarterly Report 11. Additional data, showing just perfusate extraction results, were Faxed December 28, 1994. Analysis Report Hal/Lprb 93-7 is in preparation. Remaining samples were returned to WRAIR on July 25, 1995.

Hal/P 95-4
(racemic subject 17-21 and chiral analytical data is presented in Appendix A)

Samples are to be analyzed in accordance to the protocol titled "Halofantrine as Prophylaxis against Malaria: Multiple-Dose Safety, Tolerance and Pharmacokinetics Study." Final data on 1060 samples for halofantrine and WR 178,460 (free base) concentrations were Faxed to the COR on January 3, 1997. Final results on 305 samples received on June 10, 1997 were Faxed to the COR on November 24, 1997. Results on the chiral assay of the same samples were Faxed to the COR on May 4, 1998. Analysis Report Hal/BP 95-4 is in preparation.

Hal/B 96-1
(analytical data is presented in Appendix A)

Monkey blood samples (165) to be analyzed for halofantrine were received on June 4, 1996. No protocol is available for this study. A draft report on the validation of an assay and routine analysis for halofantrine and WR 178460 in monkey blood samples was submitted for review August 19, 1997. A final report with responses to changes requested by the COR in a November 3, 1997 FAX was submitted January 23, 1998.

MEFLOQUINE

Mef/P 97-2
(analytical data is presented in Appendix A)

Analysis of 14 human plasma samples for determination of the free base concentration of mefloquine hydrochloride (WR 142,490), was accomplished by use of the HPLC method described in Study Report 14B dated August 29, 1989 under contract DAMD17-86-C-6150. Final mefloquine concentrations on 14 of 15 human plasma samples (15th=NS) were submitted in a fax dated November 13, 1997. Final primaquine, chloroquine and metabolite concentrations on 2 human plasma samples were submitted in a fax dated December 11, 1997. The samples were obtained from the Division of Experimental Therapeutics, Walter Reed Army Institute of Research.

WR 238,605

WR5/P 93-4

Results of the analysis of 330 dog plasma samples received on 7/14/93 were reported in Routine Analysis Report WR5/P 93-4, which was submitted for review on April 25, 1994. The protocol for this study is titled "Thirteen week oral toxicity study of WR 238605 with a thirteen week recovery period in dogs." Study Report 13B, Supplement II for dog plasma validation, modified as requested in a COR letter dated January 31, 1995, mandates changes in this report. Sample results were discussed in an April 3, 1995 site visit meeting and in correspondence from the COR dated April 22, 1994 and May 26, 1994. No further changes are required by the COR (July 27, 1998 fax) to finalize the report.

Analysis of 330 dog plasma samples for determination of the free base concentration of WR 238,605 was accomplished by use of an HPLC method described in Study Report 13 Dog Plasma Assay Supplement, dated April 11, 1994 and developed under contract DAMD17-92-C-2028. The samples were obtained from the University of Illinois at Chicago, in accordance with the protocol titled "Thirteen Week Oral Toxicity Study of WR 238605 with a Thirteen Week Recovery Period in Dogs." Analytical results are presented in the report dated April 25, 1994, for plasma samples from male and female dogs from analyses performed from September 30, 1993 through January 11, 1994.

WR5/P 93-5
(analytical data was presented in the DAMD17-92-C-2028 final report)

Analysis of 154 rat plasma samples for determination of the free base concentration of WR 238,605 was accomplished by use of an HPLC method described in Study Report 13 Rat Plasma Assay Supplement, dated January 20, 1994 and developed under contract DAMD17-92-C-2028. The samples were obtained from the University of Illinois at Chicago, in accordance with the protocol titled "Thirteen Week Oral Toxicity Study of WR 238605 with a Thirteen Week Recovery Period in Rats." Analytical results are presented in the report dated January 20, 1994, as amended November 4, 1996, for plasma samples from male and female rats from analyses performed from October 12 through 21, 1993. The report and amendment were accepted as final by the COR in a July 27, 1998 fax.

WR5/P 93-8
(analytical data was presented in the DAMD17-92-C-2028 final report)

Routine analysis of 359 human plasma and 359 human blood samples was completed for Analysis Report WR5/BP 93-8 for samples received in accordance with the protocol titled "Pharmacokinetics, Pharmacodynamics, Safety and Tolerance of a Single Oral Dose of WR 238605 Succinate." Final data was attached to Quarterly Report 11. Repeat analysis of selected samples, as

requested in a FAX from the COR dated December 9, 1994, was completed and results were faxed to the COR February 7, 1995. A report is in preparation.

WR5/P 94-1
(analytical data was presented in the DAMD17-92-C-2028 final report)

Monkey blood (12) samples were received September 15, 1994. Final analytical results were faxed to the COR on 11/22/94. A brief letter reporting results and referring to the human validation report as suggested by the COR at the April 3, 1995 site visit is in preparation. The analysis was set to proceed with use of blank human plasma for standard curve and control samples and blank monkey plasma as duplicate controls.

WR5/P 94-4
(analytical data was presented in the DAMD17-92-C-2028 final report)

Human plasma (28) and human blood (28) samples were received October 26, 1994 and assayed in accordance with the protocol titled "Evaluation of WR 238605 as a Prophylactic Agent against Induced *P. falciparum* Malaria Infection in Healthy Non-immune Subjects: A Dose Ranging Study." Final analytical results were faxed to the COR on 11/21/94 and the report is in preparation.

WR5/P 94-7
(analytical data was presented in the DAMD17-92-C-2028 final report)

Final results for 709 human plasma samples assayed in accordance with the protocol titled "A Multiple Dose Safety, Tolerance and Pharmacokinetic Study of WR 238605 when given to Healthy Male and Female Subjects" were Faxed to the COR on 7/22/96. Draft Analysis Report WR5/P 94-7 was submitted August 29, 1996 to the COR for review. No further changes were required by the COR (July 27, 1998 fax) and a final report was submitted August 27, 1998.

WR5/P 95-1
(analytical data was presented in the DAMD17-92-C-2028 final report)

Samples were analyzed in accordance with the protocol titled "Six Month Oral Toxicity Study of WR238605 Succinate in Rats." Rat plasma samples (325) were received September 21, 1995 and (80) January 30, 1996. Final results were faxed to the COR on September 12, 1996 (enclosed). Draft Analysis Report WR5/P 95-1 was submitted September 17, 1996 to the COR for review. The final report was submitted on July 24, 1998.

WR5/P 95-2
(analytical data is presented in Appendix A)

A draft report was submitted September 5, 1997 for WR 238605 in 226 human plasma and 226 blood and for chloroquine and monodesethyl-chloroquine in 67 human blood samples analyzed in accordance to the protocol titled "Evaluation of WR 238605 as a Prophylactic Agent against Induced *P. falciparum* Malaria Infection in Healthy Non-Immune Subjects II: A Multiple-Dose Causal versus Suppressive Study." A request by the PI for reassay of 8 plasma and 3 blood samples for WR 238605 determinations was made in a fax dated December 8, 1997 and results were submitted February 9, 1998. Results were faxed to the COR on April 23, 1998 on reassay of samples requested by the PI of an additional 29 samples for WR 238605 determinations in a COR visit on February 18, 1998. Changes requested by the COR (July 27, 1998 fax) were incorporated in the final report submitted August 28, 1998.

A WR5/P 95-2 Chiral draft report was submitted September 30, 1997 for the routine analysis of 226 human plasma samples for determination of R and S isomer WR 238605 concentrations. No further changes were required by the COR (July 27, 1998 fax) and a final chiral report was submitted September 8, 1998.

WR5/P 95-3
(analytical data is presented in Appendix A)

Dog plasma samples were analyzed in accordance to the protocol titled "WR 238605 Multiple Drug Interaction Study in Beagle Dogs" as requested in a COR letter dated October 20, 1995. Results on 131 samples (and 18 dosing solutions not reported) received April 25 and November 25, 1996 for WR 238,605, mefloquine, chloroquine, monodesethyl-chloroquine, didesethyl-chloroquine, quinine, doxycycline, halofantrine and/or WR 178460 were faxed to the COR on March 20, 1997. On January 13-14, 1998 an additional 431 plasma samples and 21 vials of dosing solutions were received. On February 10, 1998 an additional 522 plasma samples and 6 vials of dosing solutions were received. Preliminary results of analysis of these additional samples were submitted with Quarterly Report 4 dated August 26, 1998.

WR5/P 96-2
(analytical data is presented in Appendix A)

Samples are to be analyzed as requested in a COR letter dated May 6, 1996 in accordance with the protocol titled "Dose-Ranging Study of the Safety and Efficacy of WR 238605 in the Prevention of Relapse of *Plasmodium vivax* Infection in Thailand." Final results on 266 human plasma and 260 human blood samples for WR 238,605 were faxed to the COR on March 6, 1997. On December 17, 1997, an additional 357 human plasma and 358 human blood samples were received (a recount shows that 1 sample less was received than originally believed). On March 2, 1998, final WR 238,605 results on 292 plasma and 292 blood (excluding

samples from subjects dosed only with chloroquine) were faxed to the COR. Chloroquine blood and plasma analyses have been run and data is being evaluated for release. Additional samples are expected (COR fax dated March 5, 1998).

WR5/P 97-1
(analytical data is presented in Appendix A)

Samples were to be analyzed in accordance with the protocol titled " One Year Oral Toxicity Study of WR 238605 Succinate in Dogs." Samples (224 dog plasma) were received August 13, 1997. Final data was faxed to the COR September 26, 1997 and a report is in preparation.

WR 6026, WR 211,789 and WR 254,421

WR6/PU 94-3
(analytical data is presented in Appendix A)

Samples are for routine analysis in accordance with the protocol titled "Clinical Trial of Oral WR6026•2HCl in Patients with Brazilian Visceral Leishmaniasis due to *L. chagasi*: Initial Dose Range Determination for Efficacy, Safety and Tolerance." Final data on 92 human plasma and 90 human urine samples was Faxed to the COR on January 27, 1997 and on 28 human plasma samples was Faxed to the COR on August 22, 1997. On November 13, 1997, 92 human sera samples were received and a request for analysis of previously received plasma/sera samples was made for WR 254421 determinations.

GENTAMICIN AND PAROMOMYCIN

Gnt/p 96-3
(analytical data was presented in the DAMD17-92-C-2028 final report)

Samples are to be analyzed in accordance with the protocol titled " Irritant and Phototoxicity Reactions to the Topical Antileishmanial WR 279396: A Randomized, Double-Blind Phase I Study." Final data on 83 human plasma samples was faxed to the COR on December 18, 1996.

CONCLUSIONS

Using the procedures described in this report, we were able to work sequentially or simultaneously on development, validation and characterization of assays for WR 238,605 (and its stereoisomers), halofantrine (and its metabolite and their stereoisomers), WR 6026 (and its metabolites), mefloquine (and its metabolite), artelinic acid, *p*-aminoheptanophenone (and related compounds), gentamicin and paromomycin, pyridostigmine, WR 242511, chloroquine (and its metabolites), WR 243,251, quinine and doxycycline. Work on routine analyses of biological specimens during this period was performed for studies that required determination of concentrations of WR 238,605 (and its stereoisomers), halofantrine (and its metabolite and their stereoisomers), WR 6026 (and its metabolites), mefloquine, *p*-aminoheptanophenone (and related compounds), primaquine, gentamicin and paromomycin, chloroquine (and its metabolites), quinine, and doxycycline. We worked on demonstrating sensitivity, specificity, linearity, lack of interferences, accuracy, and reproducibility of the analytical method, describing the extent of recovery for the method, and reporting on the stability of compounds of interest in specimens during storage and drug analysis to provide documentation in support of Investigational New Drug (IND) submissions to the Food and Drug Administration (FDA).

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APPENDIX A

LABORATORY METHODOLOGY FOR WR 238605 *R & S* ENANTIOMERS HUMAN PLASMA ASSAY,* STUDY REPORT 27

A. INSTRUMENTS

1. Waters Intelligent Sample Processor Model 710B (Waters Associates, Milford, MA) or equivalent.
2. LC-10AS Shimadzu Pump (Shimadzu Corp., Kyoto, Japan) or equivalent.
3. Shimadzu RF-535 Fluorescence Detector (Shimadzu Corp., Kyoto, Japan) or equivalent.
4. Hewlett-Packard Reporting Integrator #3390A (Hewlett-Packard Co., Santa Clara, CA) or equivalent.

B. REAGENTS

1. WR 238605, bottle no. BK 73252 (WRAIR, Washington D.C.).
2. WR 211789 (Internal Standard), bottle no. BK 50713 (WRAIR, Washington D.C.).
3. WR 280407 (R-(-) isomer), bottle no. BN58938 (WRAIR, Washington D.C.).
4. WR 280408 (S-(+) isomer), bottle no. BN58929 (WRAIR, Washington D.C.)
5. Methyl *t*-butyl ether, HPLC grade (Baxter, Burdick & Jackson, Muskegon, MI).
6. Acetonitrile, HPLC grade (Fisher Scientific, Fair Lawn, NJ).
7. Methanol, HPLC grade (Fisher Scientific, Fair Lawn, NJ).
8. Water, Type 1 reagent grade, (deionized by Nanopure II, Barnstead Co., Boston, MA).
9. Perchloric acid, reagent grade (Fisher Scientific, Fair Lawn, NJ).
10. Sodium hydroxide, reagent grade (Fisher Scientific, Fair Lawn, NJ).

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* Minor alterations may have been made in the assay process, depending on instrument and assay conditions.

C. ASSAY CONDITIONS

1. DETECTOR

   Settings

   Wavelengths: excitation; 375 nm; emission; 480 nm
   Range: 32
   Sensitivity: high
   Response: medium

2. COLUMN

   Chiralcel OD-R, cellulose tris on a 10 µm silica gel substrate, 4.6 x 250 mm (Chiral Technologies, Inc., Exton, PA).

3. SOLVENT SYSTEM

   CH_3CN/1.0M $NaClO_4$ (45:55, v/v), adjust to *p*H 5 with perchloric acid

4. FLOW RATE

   0.5 ml/min

5. STOCK SOLUTIONS - Solutions were stored in a 4°C and checked for deterioration by comparison to a newly made solution (solutions are discarded when a more than 10% change in the absolute peak height is observed or by 6 months after the preparation date).

   a. WR 238605 succinate for precision expressed as the free base concentration.

Prep date: 7/5/96

Solution Type	Weight of Standard (mg)	Conversion Factor*	QS Volume (ml)	Solvent	Conc. (µg/ml)
Standard Curve	3.203	0.7971	25.542	50% CH_3CN	100
Control	3.203	0.7971	25.542	50% CH_3CN	100

*= Molecular weights of WR 238605 free base/WR 238605 (as succinate)

   b. WR 211789 dihydrochloride hemihydrate expressed as the free base concentration- Internal standard for precision.

Prep date: 5/13/96

Solution Type	Weight of Standard (mg)	Conversion Factor*	QS Volume (ml)	Solvent	Conc. (µg/ml)
Internal std.	2.729	0.7937	25	50% CH_3CN	86.5

*= Molecular weights of WR 211,789 free base/WR 211,789•2HCl•1/2H_2O

6. WORKING SOLUTIONS - Solutions were stored in a 4°C refrigerator and discarded when stock solutions were discarded or by 6 months after the preparation date.

   a. WR 238605 succinate expressed as the free base concentration.

Solution Type	Conc. Diluted (µg/ml)	Dilution Ratio (ml)	Solvent	Conc. (µg/ml)
Standard Curve	100	1:10	50% CH_3CN	10.0
Control	100	1:10	50% CH_3CN	10.0

   b. WR 211789 dihydrochloride hemihydrate expressed as the free base concentration - Internal standard. The stock solution is the working solution.

7. RETENTION TIMES (subject to change depending on temperature and column performance).

   a. WR 238605 - (+) peak; 40 min and (-) peak: 42.5 min.
   b. WR 211789 (Internal Standard) - 25 min

8. BLANK PLASMA

   Human plasma (CPD or CPDA-1 as anticoagulant) was obtained from the San Francisco Irwin Memorial Blood Bank.

9. INJECTION VOLUME

   10-100 µl

10. QUANTITATION

    By peak height ratio of drug peak relative to internal standard peak. Standard curves are calculated by weighted linear regression where weights = 1/y.

11. LOWER LIMIT OF QUANTITATION OF METHOD (The lower limit of quantitation of the assay of human plasma for WR 238605 R & S enantiomers was based on the interday and intraday low point validation results, on standard curve calibrator results, and a minimum 3 to 1 signal to noise ratio.)

    5.00 ng/ml WR 238605 R & S enantiomers in plasma.

12. VOLUME MEASUREMENT

    Plasma sample volumes were measured with a 1000 µl Gilson

Pipetman. Hamilton syringes were used to measure standard and control solution volumes.

13. WISP OPERATING TEMPERATURE

    Room temperature.

D. SAMPLE STORAGE

All samples were kept frozen at -20°C before analysis and thawed for preparation and analysis, unless specified otherwise.

E. SAMPLE PREPARATION

1. Pipet 1.0 ml of human plasma into a culture tube.
2. Spike standard curve samples as shown in Section G "Generation of Standard Curve Calibrators."
3. Thaw frozen samples in cold water or at room temperature.
4. Vortex for 1 min.
5. Add 75 µl of internal standard working solution (86.5 µg/ml) and vortex for 10 seconds.
6. Add 0.1 ml of 1M NaOH and vortex for 10 seconds.
7. Add 3 ml of methyl *t*-butyl ether and vortex for 2 min.
8. Centrifuge for 10 min at ~2000 *g* and transfer the organic layer to a second tube.
9. Repeat steps 7 and 8, combining supernatants.
10. Evaporate to dryness under nitrogen.
11. And reconstitute with 400 µl of mobile phase for injection onto column.

F. QUALITY CONTROL

1. Content and frequency of blanks

   A blank plasma sample was prepared as described in "Sample Preparation" and assayed at least once for each standard curve in precision assays.

2. PIPETTE CALIBRATION

   SOP 2C-1.2: "Pipet calibration is done for every new study. If the study's duration is a month or less, it is up to the discretion of the study leader to re-calibrate. For studies that take more than two months, calibration must be done every month."

   "Pipet water into weighed container or tray and write weight of water that the pipet holds. Do a series of six individual measurements on the low, medium and high volume ranges as specified or recommended by the manufacturer. Calculate the mean, standard deviation, % Error, and % CV."

   "Acceptable % CV and % Error must be equal or less than 2.0 %."

3. BALANCE CALIBRATION: Balance calibration is performed yearly by the Bay City Scale Co., Burlingame, CA. Balance calibration is also performed, in house, according to SOP 2C-2.1.

   SOP 2C-2.1: "The balance calibration procedure must be performed on balances used to weigh compounds for use as standards (e.g. the Sartorius balance, SSF). The First Use Calibration Procedure must be performed daily, by the first person to use the balance that day. The full range calibration procedure will be performed on a bimonthly basis by assigned personnel.

   First Use Calibration Procedure:

   1. Follow the Full Range Calibration Procedure, but calibrate with two weights only, to cover the range of normal weighings (e.g. for the Sartorius balance, SSF, in the semimicro range (30.0 g) with the 5 mg and 20 mg standard weights).
   2. If readings fall out of acceptable limits perform the Full Range Calibration Procedure.

   Full Range Calibration Procedure:

   1. Use the set of standard weights to calibrate the balance.
   2. Use weights to test the full range of the balance (e.g. for the Sartorius balance, SSF, use semimicro range (30.0 g) for standard weights equal to or less than 200.0 mg and macro range (160.0 g) for weights greater than 200.0 mg; and test the weights shown in the following table)...."

   Standard Weights of 2, 5, 10, 100, and 2000 mg were used.

"5. Take four readings of each weight and calculate the average, standard deviation, percent error, and percent coefficient of variation.
6. Percent error, and percent coefficient of variation must be equal to or less than 2.0%."

G. GENERATION OF STANDARD CURVE CALIBRATORS

Calibration standards were generated by spiking 1.00 ml blank human plasma specimens with WR 238605 standard curve solutions. This procedure is equivalent to addition of the R and S isomer masses of WR 238605 shown below. Since 1.00 ml plasma samples are assayed, these amounts correspond to the nominal concentrations shown below.

Generation of WR 238605 Standard Curve Calibrators

Sample	Volume Spiked (µl)	Spiking Solution Concentration (µg/ml)	Mass Spiked (ng)	Standard Curve Sample Nominal Concentration (ng/ml)
00*	0	0	0	0
0**	0	0	0	0
1	1	10.0	5.00	5.00
2	2	10.0	10.0	10.0
3	4	10.0	20.0	20.0
4	8	10.0	40.0	40.0
5	15	10.0	75.0	75.0
6	30	10.0	150	150
7	6	100	300	300
8	12	100	600	600
9	20	100	1000	1000

H. GENERATION OF PRECISION SAMPLES

Quality control samples were generated by spiking 1.00 ml blank human plasma specimens as follows.

Generation of WR 238605 Precision Samples

	Volume Spiked (µl)	Spiking Solution Concentration (µg/ml)	Plasma Volume (ml)	Quality Control Sample Nominal Concentration (ng/ml)
X-Lo	2.00	10.0	1.00	10.0
Low	10.0	10.0	1.00	50.0
Med.	4.00	100	1.00	200
Hi	16.0	100	1.00	800

* 00 = Sample with no drug and no internal standard.
** 0 = Sample with no drug but with internal standard.

I. GENERATION OF RECOVERY SAMPLES

WR 238605 recovery from plasma extraction was assessed at four different concentrations by comparing the WR 238605 to internal standard peak height ratios in reference samples to the peak height ratios in plasma. Plasma (1 ml) samples were spiked with WR 238605 succinate (and vortexed) then prepared as described above in "Sample Preparation," except no standard curve was used, 5 ml of supernatant was taken for evaporation, and injection volume ranged 5-40 µl. Reference samples were generated by spiking drug and internal standard into 6 ml of methyl *t*-butyl ether, vortexing, evaporating 5 ml of the solution and reconstituting in mobile phase. Reference sample injection volume ranged 5-40 µl.

J. VALIDATION RESULTS

1. STANDARD CURVE

   Chromatograms for each point in representative standard curve for the R and S isomers of WR 238605 appear in Figure 4. Peak height ratios for these calibrators appear in Table 1. Statistical parameters of human plasma interday precision standard curve calibrators appear in Table 2.

2. INTRA- AND INTERDAY PRECISION

   Results for these evaluations appear in Table 3 for human plasma.

3. LLOQ

   Results for this evaluation appear in Table 4 for human plasma.

4. RECOVERY

   Results for this evaluation appear in Table 5 for human plasma.

Validation of a Liquid Chromatographic/Mass Spectrometric/Mass Spectrometric (LC/MS/MS) Method for the Determination of Chloroquine and Monodesethyl-chloroquine in Human Blood Samples

III. Methods

A. Materials

Test Compounds:	Chloroquine Monodesethyl-chloroquine
Drug Standards:	Monodesethyl-chloroquine (WR 029623), Bottle number BL11088, was obtained from WRAIR. Chloroquine (WR 1544), Bottle number AU29291, was obtained from WRAIR.
Internal Standard:	Neostigmine bromide, Lot number KT05130JT, was purchased from Aldrich Chemical Co, Milwaukee, WI.
Matrix:	Human Blood
Biological Matrix:	Control and standard curve human blood was obtained from Irwin Memorial Blood Bank, San Francisco, CA. This matrix was used for daily preparation of standard and quality control samples. Prior to use, blood was stored at -20°C. This material was found to be free of endogenous substances that would interfere with the quantitation of the drug, metabolite or internal standard.
Sample Storage:	Temperature: Approximately -20°C

LC/MS/MS System

Detector:	API III PE-Sciex (Perkin-Elmer, Norwalk, CT)
Pump:	Shimadzu LC-10AD Pump (Shimadzu Scientific Instruments, Inc., Columbus, MD) or equivalent
Injector:	Waters Intelligent Sample Processor 717 Plus (Waters Associates, Milford, MA) or equivalent
Data Acquisition:	Macintosh Quadra 800 (Apple, Cupertino, CA) Mac Spec 3.3 Software (Perkin-Elmer, Norwalk, CT) RAD 2.4 Software (Perkin-Elmer, Norwalk, CT)
Data Reduction:	Internal standard method using peak area ratio (PAR). Weighted (1/y) linear regression of concentration (x-axis) vs. PAR (y-axis)

LC/MS/MS Conditions

Column:	Hypersil Silica, 3 µm particle size, 4.6 X 50 mm (Keystone Scientific, Inc., Bellefonte, PA) or equivalent
Column Temperature:	Room Temperature
Mobile Phase:	90% acetonitrile, 0.1% trifluoroacetic acid (TFA), 5 mM ammonium acetate. The mobile phase was prepared by mixing 3600 ml of CH_3CN with 400 ml of water, adding 4 ml of TFA and adding 10 ml of 2M NH_4CH_2COOH to yield approximately 4 liters. The resulting solution was filtered through a 5 micron filter and degassed under vacuum prior to use.
Flow Rate:	1.2 ml/min.
Sample Inlet Mode:	Heated Nebulizer
Ionization Mode:	APCI/Positive Ionization
Discharge Current:	+ 3 µA
Curtain Gas Flow Rate:	1.2 L/min (N_2 = 99.999%)
Nebulizer Pressure:	80 psi (Ultra Zero Air)
Auxiliary Flow Rate:	2.0 L/min (Ultra Zero Air)
CAD Gas:	250 x 10^{12} molecules/cm^2 (9.99% N_2/Ar)
Interface Heater Temperature:	55°C
Heated Nebulizer Temperature Controller:	480°C-500°C

Note: If necessary, the LC/MS/MS conditions can be slightly modified to optimize the system.

Mass Scanning Mode: MRM (Multiple Reaction Monitoring)

Chloroquine:	321 - 247 *m/z*
Monodesethyl Chloroquine:	292 - 114 *m/z*
Internal Standard:	209 - 72 *m/z*

Assay Parameters

Volume of Blood Required for Assay:	100 µl
Assay Ranges:	Chloroquine and Monodesethyl Chloroquine: 20.0 to 2000 ng/ml
Minimum Reportable Concentrations:	20.0 ng/ml for Chloroquine and Monodesethyl Chloroquine

Chemicals and Supplies

Chemical/Solvents	Grade	Supplier
Acetonitrile	HPLC	Fisher Scientific
Ammonium acetate	HPLC	Fisher Scientific
Trifluoroacetic Acid	Reagent	Sigma Chemical
Acetic Acid	Reagent	Fisher Scientific
Water	Type I Reagent Grade	Nanopure, Barnstead
Methanol	Optima	Fisher Scientific

B. Analytical Method

Human blood samples (100 µl) were analyzed for chloroquine and mono-desethyl-chloroquine with an LC/MS/MS procedure in a PE Sciex-API III system equipped with a silica column (4.6 x 50 mm, 5 µm particle size), 90% CH_3CN, 0.1% trifluoroacetic acid (TFA) and (final concentration) 5 mM ammonium acetate mobile phase and mass spectrometric detection with sample inlet by heated nebulizer, positive ionization by APCI (atmospheric pressure chemical ionization) and mass scanning by MRM (Multiple Reaction Monitoring) analysis. Sample preparation consisted of addition of 50 µl of water, sonication of the mixture, addition of neostigmine bromide internal standard (IS), and transfer of the supernatant prior to separation by LC/MS/MS. Standard curve and quality control (QC) samples were generated by spiking interference free human blood samples with known amounts of chloroquine, monodesethyl-chloroquine, and IS. Standard curve, QC and assay samples were prepared as described, then 1-2 µl aliquots were injected into the LC/MS/MS system for chromatographic separation and subsequent mass spectrometric detection. The peak area ratios of chloroquine and monodesethyl-chloroquine to IS were calculated for each sample from the measured peak areas obtained by LC/MS/MS. Finally, spiked concentrations and chloroquine and mono-desethyl-chloroquine to IS peak area ratios of the standard curve samples were fit by 1/y weighted least squares linear regression to the two equations for the best straight lines ($y = mx + b$, where y = peak area ratio and x = chloroquine or monodesethyl-chloroquine concentrations), and drug and metabolite concentrations in assay samples were calculated by these equations from the chloroquine and monodesethyl-chloroquine to IS peak area ratios obtained by LC/MS/MS.

Calibration standards and validation samples with drug concentrations within the calibration range of the assay were analyzed to assess the performance of the assay. Calibration standards and validation samples were generated by spiking blank human blood with chloroquine and monodesethyl-chloroquine. A didesethyl-chloroquine stock solution was also generated, diluted into working solutions and spiked into validation samples but data is not presented in this report, since validation acceptance criteria were not met.

Standard and Control Solutions

STOCK SOLUTIONS: These solutions were stored in a 4°C refrigerator. The chloroquine (CHL) and monodesethyl-chloroquine (MDC) were protected from light.

Solution Type	Weight of Standard (mg)	Conversion Factor	QS Volume (ml)	Solvent	Conc. (mg/ml)
CHL Standard Curve	1.71	0.6200*	10.0	50% CH_3OH	0.106
MDC Standard Curve	1.77	0.6185*	10.0	50% CH_3OH	0.109
CHL Control	1.86	0.6200*	10.0	50% CH_3OH	0.115
MDC Control	1.66	0.6185*	10.0	50% CH_3OH	0.103
Neostigmine Internal Standard	1.018	0.982	100	50% CH_3OH	0.100

* = Molecular weights of chloroquine free base/chloroquine diphosphate or monodesethyl-chloroquine free base/monodesethyl-chloroquine dioxalate;

WORKING SOLUTIONS. These solutions were stored in a 4°C refrigerator and protected against light (neostigmine solution was not protected). CHL and MDC stock solutions were combined into a single solution (Solution A), diluted with 50% methanol and QS to 10.0 ml to make 5.00 µg/ml CHL and MDC concentrations. The low working solutions were generated with a 40% methanol, 40 mM ammonium acetate, 0.1% acetic acid diluting solution

Solution Type	Volume Diluted (ml)	Conc. Diluted (µg/ml)	QS Volume (ml)	Solvent	Conc. (µg/ml)
Standard Curve Solution A [CHL]	0.472	106	10.0	50% CH_3OH	5.00
Standard Curve Solution A MDC]	0.458	109	10.0	50% CH_3OH	5.00
Low Working Standard Curve Solution [CHL and MDC]	1.00	5.00	10.0	diluting solution	0.500
Control Solution A [CHL]	0.434	115	10.0	50% CH_3OH	5.00
Control Solution A [MDC]	0.488	103	10.0	diluting solution	5.00
Low Working Control Solution [CHL and MDC]	1.00	5.00	10.0	50% CH_3OH	0.500
Neostigmine Internal Standard	0.100	100	100	CH_3OH	0.100

Calibration Standards and Quality Control Samples

The scheme for generating calibration standard and quality control (QC) samples for chloroquine and monodesethyl-chloroquine is provided in the following tables.

Calibration Standards: Calibration standards were generated by spiking 0.100 ml blank human blood specimens with chloroquine and monodesethyl-chloroquine standard curve solutions. This procedure is equivalent to addition of the masses of chloroquine and monodesethyl-chloroquine shown below. Since 0.100 ml blood samples are assayed, these amounts correspond to the nominal concentrations shown below. Vortex for 10 seconds.

Generation of Chloroquine and Monodesethyl-chloroquine Standard Curve Calibrators

Sample	Volume Spiked (µl)	Spiking Solution Concentration (µg/ml)	Mass Spiked (ng)	Standard Curve Sample Nominal Concentration (ng/ml)
00*	0	0	0	0
0**	0	0	0	0
1	4.00	0.500	2.00	20.0
2	8.00	0.500	4.00	40.0
3	16.0	0.500	8.00	80.0
4	30.0	0.500	15.0	150
5	6.00	5.00	30.0	300
6	10.0	5.00	50.0	500
7	20.0	5.00	100	1000
8	40.0	5.00	200	2000

Quality Control Samples: Quality control samples were generated by spiking 100 µl blank human blood specimens as follows.

Generation of Chloroquine and Monodesethyl-chloroquine Precision Quality Control Samples

	Volume Spiked (µl)	Spiking Solution Concentration (µg/ml)	Blood Volume (µl)	Quality Control Sample Nominal Concentration (ng/ml)
X-Lo	8.00	0.500	100	40.0
Low	20.0	0.500	100	100
Med.	5.00	5.00	100	250
Hi	30.0	5.00	100	1500

* 00 = Sample with no drug and no internal standard.

** 0 = Sample with no drug but with internal standard.

SAMPLE PREPARATION PROCEDURE

1. Pipet 100 µl of blank human blood into a 13 x 100 tube.
2. Spike standard curve samples with chloroquine and monodesethyl-chloroquine as described above.
3. Let stand at room temperature for 30 minutes to equilibrate.
4. Add 50 µl of water.
5. Sonicate the mixture for 5 minutes to lyse cells.
6. Add 400 µl of acetonitrile/internal standard working solution (100 ng/ml neostigmine) and vortex 1 minute.
7. Centrifuge for 5 minutes at 2000 x *g*.
8. Transfer supernatant to autosampler vial and inject 1-2 µl onto the LC column.

APPENDIX B

Routine Assay Data

Subject	Time	Plasma WR6026 (ng/ml)	Plasma WR211789 (ng/ml)	Urine WR6026 (ng/ml)	Urine WR211789 (ng/ml)	Urine WR254421 (ng/ml)
1A	WK-1	*	*	*	*	*
	Day 7	63.2	43.3	215	82.4	1510
1B	WK-1	*	*	*	*	*
	Day 7	8.29	11.9	264	96.4	1380
	Day 14	9.11	12.1	191	62.1	831
	Day 21	7.94	11.1	245	88.1	1000
	Day 28	6.66	10.1	70.9	59	468
	Day 42	*	*	*	*	*
1C	WK-1	*	*	*	*	*
	Day 7	45.1	64.2	115	45.7	824
	Day 14	33	61.1	68.8	36.3	610
	Day 21	28.5	56.4	24.3	16	271
	Day 28	28.5	54.6	*	*	54.3
	Day 35	*	*	ns	ns	ns
1D	WK-1	*	*	*	*	*
	Day 7	33.7	34.7	163	54.8	2350
	Day 14	37.4	40.1	37.2	15.2	522
	Day 21	29.5	39.1	22.6	11.6	474
	Day 28	26.1	33.8	9.33	8.09	235
	Day 42	*	*	*	*	*
2A	WK-1	*	*	*	*	*
	Day 7	197	124	636	276	1510
	Day 14	30.3	56.4	590	267	1360
	Day 21	63.9	90.4	1830	872	2990
	Day 28	58.1	97.5	(day 31) 28.5	(day 31)14.0	(day 31)73.2
	Day 42	*	*	*	*	*
2B	WK-1	*	*	*	*	*
	Day 7	362	102	697	189	1830
	Day 14	230	108	2320	1000	4390
	Day 21	71.4	90.4	464	181	1790
day 30	Day 28	41.8	70	(day 30)117	(day 30)114	(day 30)661
	Day 42	*	*	*	*	*
2C	WK-1	*	*	*	*	*
	Day 7	38.3	51.9	29	10.6	487
	Day 14	29.5	49	40.4	25.2	716
	Day 21	24.2	31.5	122	30.1	856
	Day 28	15.2	26.2	7.28	9.2	132
	Day 42	*	*	*	*	*

ns = no sample; * = below assay sensitivity (1.00 ng/ml in plasma, 5 ng/ml for WR 6026 and WR 211789 and 50 ng/ml for WR 254421 in urine); Numbers in parenthesis indicate rescheduled day.

Subject	Time	Plasma WR6026 (ng/ml)	Plasma WR211789 (ng/ml)	Urine WR6026 (ng/ml)	Urine WR211789 (ng/ml)	Urine WR254421 (ng/ml)
2D	WK-1	*	*	*	*	*
	Day 7	198	92.1	36.5	21.7	641
	Day 14	135	67.1	357	111	2430
	Day 21	85.2	52.8	264	82.7	2400
	Day 28	52	41	27.6	28.9	680
	Day 31	3.87	1.33	ns	ns	ns
2E	WK-1	*	*	*	*	*
	Day 7	956	362	828	578	252
	Day 14	671	300	1510	710	276
	Day 21	563	191	1720	740	452
	Day 28	661	220	577	262	165
	Day 42	3.89	*	16.6	*	*
2F	WK-1	*	*	*	*	*
	Day 7	56.8	56.9	246	137	1480
	Day 14	64.7	51.5	633	319	1850
	Day 21	53.8	40.5	707	339	2500
	Day 28	44.1	36.1	139	148	967
	Day 42	*	*	*	*	*
3A	WK-1	*	*	*	*	*
	Day 7	72.4	58.3	505	286	6060
	Day 14	39.9	33.4	539	282	3820
	Day 21	34.4	24.9	245	82.3	2730
	Day 28	43.7	30	209	159	2490
	Day 42	*	*	*	*	67.3
3B	WK-1	*	*	*	*	*
	Day 7	142	84.2	366	228	2000
	Day 14	106	81.6	403	397	2560
	Day 21	136	95.5	455	223	1620
	Day 28	271	146	177	164	1460
	Day 42	*	*	*	*	*
3C	WK-1	*	*	*	*	*
	Day 7	185	144	443	250	1580
	Day 14	115	108	915	753	1780
	Day 21	112	90.8	2260	1230	2830
	Day 28	67	56.6	238	229	1250
	Day 42	*	*	*	*	*

ns = no sample; * = below assay sensitivity (1.00 ng/ml in plasma, 5 ng/ml for WR 6026 and WR 211789 and 50 ng/ml for WR 254421 in urine); Numbers in parenthesis indicate rescheduled day.

Subject	Time	Plasma WR6026 (ng/ml)	Plasma WR211789 (ng/ml)	Urine WR6026 (ng/ml)	Urine WR211789 (ng/ml)	Urine WR254421 (ng/ml)
3D	WK-1	*	*	*	*	*
	Day 7	370	118	792	295	2190
	Day 14	245	109	695	258	1970
	Day 21	161	81.8	2180	740	3900
	Day 28	146	79.4	252	185	1470
	Day 42	*	*	*	*	*
3E	WK-1	*	*	*	*	*
	Day 7	150	71.9	644	305	2290
	Day 14	63.4	45.6	675	327	2120
	Day 21	61	47.9	1310	529	2920
	Day 28	61	41.3	302	200	972
	Day 42	*	*	*	*	*
3F	WK-1	*	*	*	*	*
	Day 7	267	105	189	40.7	1050
	Day 14	111	87.2	152	32.8	728
	Day 21	140	73.9	528	167	2280
	Day 28	128	62.2	108	58.2	955
	Day 42	*	*	*	*	*

ns = no sample; * = below assay sensitivity (1.00 ng/ml in plasma, 5 ng/ml for WR 6026 and WR 211789 and 50 ng/ml for WR 254421 in urine); Numbers in parenthesis indicate rescheduled day.

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WR 6026 in Human Plasma

Subject #	Time	Conc(ng/ml)
4A	Wk-1	*
	Day 7	244
	Day 14	203
	Day 21	177
	Day 28	83.7
	Day 42	2.04
4B	Wk-1	*
	Day 7	378
	Day 14	363
	Day 21	226
	Day 28	302
	Day 42	*
4C	Wk-1	*
	Day 7	132
	Day 14	118
	Day 21	122
	Day 28	119
	Day 42	1.03
4D	Wk-1	*
	Day 7	1790
	Day 14	2390
	Day 21	1690
	Day 28	1500
4E	Wk-1	*
	Day 7	85.8
	Day 14	107
	Day 21	91.9
	Day 28	65.3

WR 211789 in Human Plasma

Subject #	Time	Conc(ng/ml)
4A	Wk-1	*
	Day 7	191
	Day 14	184
	Day 21	190
	Day 28	102
	Day 42	*
4B	Wk-1	*
	Day 7	317
	Day 14	276
	Day 21	181
	Day 28	222
	Day 42	*
4C	Wk-1	*
	Day 7	144
	Day 14	106
	Day 21	96.1
	Day 28	97.3
	Day 42	*
4D	Wk-1	*
	Day 7	277
	Day 14	495
	Day 21	508
	Day 28	573
4E	Wk-1	*
	Day 7	53.7
	Day 14	101
	Day 21	76.3
	Day 28	64.6

ANALYTICAL RESULTS: WR 238605 in Human Plasma; Analytical Report WR5/BP 95-2§

Subject No.	1	2	3	5	6
Time (hr)	Concentration (ng/ml)				
0	*	*	*	*	*
0.5	*	*	*	*	5.38
1	15.8	*	3.08	*	7.13
2	44.5	18.6	35.9	8.29	87.1
4	116	130	124	115	274
8	278	230	313	315	394
12	308	237	295	500	440
16	279	267	252	363	367
24	223	190	225	339	335
36	654	486	608	1030	781
48	568	511	505	835	719
72	586	323	366	696	630
192	313	266	258	557	437
216	476	382	329	642	582
360	283	253	303	390	364
528	309	349	315	333	328
696	438	299	468	323	274
720	370	426	561	NS	NS
864	236	303	329	273	274
1032	226	187	307	207	202
1200	272	153	200	142	199
1536	207	81.3	121	97.4	130
2424	60.2	24.9	61.6	21.1	31.2

Subject No.	7	8	9	11	12
Time (hr)	Concentration (ng/ml)				
0	*	*	*	*	*
0.5	*	*	*	2.00	*
1	*	2.14	*	12.0	2.05
2	62.1	35.3	22.3	68.2	59.2
4	218	157	137	209	121
8	336	436	258	395	218
12	345	397	328	353	210
16	366	412	305	368	184
24	275	389	340	305	165
36	687	822	642	703	390
48	597	793	678	612	400
72	571	596	496	629	351
192	379	370	389	373	223
216	404	485	403	494	351
360	309	640	298	340	261
528	317	320	383	302	244
696	240	277	339	388	324
720	NS	NS	420	523	427
864	175	236	337	417	317
1032	119	230	283	341	280
1200	91.5	125	117	299	188
1536	43.7	70.8	80.4	157	156
2424	7.45	17.5	21.8	40.9	43.2

ANALYTICAL RESULTS: WR 238605 in Human Blood; Analytical Report WR5/BP 95-2§

Subject No.	1	2	3	5	6
Time (hr)	Concentration (ng/ml)				
0	*	*	*	*	*
0.5	*	*	*	*	*
1	20.7	*	6.01	*	5.68
2	85.2	48.1	66.3	18.5	175
4	318	267	217	214	412
8	718	419	430	506	650
12	770	448	465	740	735
16	764	421	409	683	622
24	653	370	378	592	611
36	1230	761	912	1470	1270
48	1430	802	725	1270	1120
72	1380	665	652	1120	983
192	895	470	460	827	716
216	1140	682	632	991	997
360	603	431	466	636	606
528	668	535	478	601	926
696	652	414	426	388	343
720	855	587	527	NS	NS
864	496	437	392	329	237
1032	375	296	314	237	213
1200	331	171	247	183	178
1536	230	153	186	106	87.0
2424	90.2	41.3	59.5	25.0	32.3

Subject No.	7	8	9	11	12
Time (hr)	Concentration (ng/ml)				
0	*	*	*	*	*
0.5	*	*	*	4.34	*
1	*	3.41	*	29.1	5.18
2	96.7	60.5	37.3	141	127
4	348	199	199	298	263
8	530	537	388	545	384
12	547	612	449	583	413
16	545	663	436	618	390
24	448	606	462	588	387
36	1010	1230	839	1650	855
48	870	1250	891	1110	779
72	835	1160	999	1010	711
192	531	731	469	711	477
216	698	945	632	850	619
360	441	936	431	508	464
528	458	478	430	663	475
696	283	241	401	442	384
720	NS	NS	657	830	360
864	178	165	294	479	489
1032	145	143	198	291	273
1200	93.5	120	85.9	279	195
1536	41.9	74.2	64.6	153	145
2424	9.71	22.1	17.9	43.1	57.1

ANALYTICAL RESULTS: Chloroquine in Human Blood; Analytical Report WR5/BP 95-2§

Monodesethyl-chloroquine

Subject 4	Concentration (ng/ml)
10/9/95	*
10/22/95	59.9
10/23/95	143
10/24/95	124
10/25/95	206
10/26/95	181
10/30/95	181
11/6/95	116
11/13/95	72.6
11/20/95	49.8
11/27/95	38.3
11/29/95	66.9

Subject 5	Concentration (ng/ml)
10/8/95	*
10/25/95	300
10/26/95	463
10/27/95	642
10/30/95	361
11/6/95	197
11/13/95	140
11/20/95	91.5
11/27/95	58.3

Subject 6	Concentration (ng/ml)
10/8/95	*
10/25/95	166
10/26/95	278
10/27/95	336
10/30/95	263
11/6/95	143
11/13/95	89.3
11/21/95	57.0
11/27/95	31.3

Subject 7	Concentration (ng/ml)
10/8/95	*
10/25/95	164
10/26/95	234
10/27/95	330
10/30/95	210
11/6/95	90.6
11/13/95	69.9
11/20/95	41.0
11/27/95	30.9

Subject 8	Concentration (ng/ml)
10/8/95	*
10/23/95	205
10/24/95	339
10/25/95	513
10/30/95	329
11/6/95	221
11/15/95	112
11/20/95	92.2
11/27/95	66.7

Subject 9	Concentration (ng/ml)
10/23/95	*
11/11/95	91.6
11/12/95	148
11/13/95	202
11/16/95	182
11/20/95	117
12/4/95	42.7
12/11/95	33.9
12/18/95	24.8

Subject 10	Concentration (ng/ml)
10/10/95	*
10/22/95	132
10/23/95	280
10/24/95	238
10/25/95	355
10/30/95	217
11/6/95	118
11/13/95	55.0
11/20/95	38.6
11/27/95	35.3

§ = Below lower limit of quantitation, 2.00 ng/ml. NS = no sample.

ANALYTICAL RESULTS: Chloroquine in Human Blood; Analytical Report WR5/BP 95-2§

Chloroquine

Subject 4	Concentration (ng/ml)
10/9/95	*
10/22/95	454
10/23/95	617
10/24/95	502
10/25/95	674
10/26/95	537
10/30/95	367
11/6/95	174
11/13/95	84.3
11/20/95	62.4
11/27/95	44.3
11/29/95	53.3

Subject 5	Concentration (ng/ml)
10/8/95	*
10/25/95	1460
10/26/95	1780
10/27/95	1680
10/30/95	669
11/6/95	263
11/13/95	140
11/20/95	78.7
11/27/95	49.4

Subject 6	Concentration (ng/ml)
10/8/95	*
10/25/95	1340
10/26/95	1630
10/27/95	1430
10/30/95	698
11/6/95	295
11/13/95	141
11/21/95	84.2
11/27/95	46.0

Subject 7	Concentration (ng/ml)
10/8/95	*
10/25/95	866
10/26/95	743
10/27/95	802
10/30/95	380
11/6/95	109
11/13/95	90.5
11/20/95	35.5
11/27/95	23.3

Subject 8	Concentration (ng/ml)
10/8/95	*
10/23/95	1250
10/24/95	1450
10/25/95	1230
10/30/95	434
11/6/95	219
11/15/95	89.9
11/20/95	86.2
11/27/95	50.5

Subject 9	Concentration (ng/ml)
10/23/95	*
11/11/95	554
11/12/95	769
11/13/95	656
11/16/95	364
11/20/95	202
12/4/95	47.1
12/11/95	28.3
12/18/95	21.2

Subject 10	Concentration (ng/ml)
10/10/95	*
10/22/95	956
10/23/95	1220
10/24/95	794
10/25/95	904
10/30/95	491
11/6/95	161
11/13/95	59.4
11/20/95	40.6
11/27/95	31.1

ANALYTICAL RESULTS: S (+) Isomer of WR 238605 in Human Plasma

Subject No.	1	2	3	5	6
Time (hr)	Concentration (ng/ml)				
0	*	*	*	*	*
0.5	*	*	5.02	*	*
1	12.0	*	5.70	*	*
2	23.0	12.2	17.4	13.6	55.8
4	59.5	56.1	59.4	52.7	143
8	139	96.2	111	176	188
12	139	82.3	112	286	196
16	123	77.8	110	210	202
24	109	66.9	102	162	162
36	249	128	271	512	412
48	288	151	196	292	404
72	240	109	212	287	312
192	139	95.2	123	271	205
216	226	105	54.3	326	274
360	68.7	77.1	136	192	174
528	132	97.3	120	155	137
696	89.3	36.3	135	165	122
720	239	71.5	156	NS	NS
864	186	91.7	121	180	125
1032	94.6	68.0	125	116	97.6
1200	137	27.5	50.3	91.8	46.4
1536	102	45.7	35.2	60.9	53.3
2424	44.2	15.7	28.2	20.7	12.4

Subject No.	7	8	9	11	12
Time (hr)	Concentration (ng/ml)				
0	*	*	*	*	*
0.5	*	*	*	*	*
1	*	*	*	9.49	*
2	19.0	17.5	10.7	31.6	42.4
4	54.1	61.4	64.8	52.1	76.8
8	107	164	115	121	124
12	115	144	139	102	128
16	161	182	131	101	102
24	96.9	186	150	63.7	98.0
36	311	288	300	196	273
48	159	300	192	363	221
72	205	220	221	168	225
192	89.2	182	182	168	146
216	143	242	219	153	210
360	108	212	153	177	121
528	112	129	187	106	139
696	82.6	146	148	129	149
720	NS	NS	207	200	164
864	71.1	90.3	174	137	153
1032	46.7	102	95.5	128	136
1200	30.1	62.7	53.1	152	95.7
1536	18.2	46.2	36.7	57.9	75.8
2424	12.9	11.6	7.09	15.6	30.8

NS=no sample.

ANALYTICAL RESULTS: R (-) Isomer of WR 238605 in Human Plasma

Subject No.	1	2	3	5	6
Time (hr)	Concentration (ng/ml)				
0	*	*	*	*	*
0.5	*	*	*	*	*
1	14.5	*	*	*	*
2	28.7	11.6	18.3	12.7	62.9
4	74.4	61.0	64.2	61.9	173
8	164	106	119	180	218
12	159	88.8	118	293	232
16	145	84.4	116	226	230
24	128	74.9	107	169	208
36	296	141	286	534	478
48	330	163	213	325	462
72	282	123	219	311	379
192	158	103	125	270	233
216	261	116	56.7	307	335
360	75.7	83.6	136	198	195
528	151	102	121	151	156
696	97.6	35.9	134	164	140
720	265	78.4	159	NS	NS
864	208	93.6	122	172	118
1032	106	70.1	122	107	94.6
1200	144	28.1	47.2	86.5	41.3
1536	105	45.4	33.2	51.8	51.3
2424	41.8	14.7	24.5	18.4	13.1

Subject No.	7	8	9	11	12
Time (hr)	Concentration (ng/ml)				
0	*	*	*	*	*
0.5	*	*	*	*	*
1	*	*	*	8.08	*
2	19.9	18.2	12.8	36.1	43.4
4	57.0	65.7	80.7	46.9	89.7
8	110	182	140	130	135
12	128	159	158	105	140
16	172	198	169	123	113
24	98.1	202	177	79.2	105
36	356	319	331	218	312
48	170	340	229	399	241
72	221	240	247	198	247
192	106	216	209	186	153
216	162	265	256	178	224
360	120	224	175	201	131
528	125	138	203	112	133
696	83.0	144	169	134	155
720	NS	NS	236	199	175
864	73.4	90.0	204	144	157
1032	51.1	99.6	112	138	135
1200	31.1	62.2	63.9	147	84.5
1536	18.3	42.2	41.9	53.7	68.7
2424	11.2	10.5	10.0	17.5	24.9

NS=no sample.

95-3.final data

3/20/97

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1695-0				*	*				
1695-0.25				793	809			*	
1695-0.5				1680	1010			7.82	
1695-1				2830	1400			84.7	
1695-2				2450	1460			195	
1695-4				1450	1040			285	
1695-6				1180	527			358	
1695-8				749	304			266	
1695-12				175	239			195	
1695-18				42.5	271			167	
1695-24				10.4	298			103	
1695-48				*	63.6			34.3	
1695-72				*	*			11.0	
1695-96				*	*			2.92	
1695-120				*	*			*	
1695-168				*	*			*	
1695-336				*	*				
1695-504				*	*				
1696-0				*	*				*
1696-0.5				*	*				333
1696-2				*	*				780
1696-4				*	*				831
1696-8				*	*				825
1696-10				*	*				776
1696-10.5				935	705				536
1696-11				1560	2200				574
1696-12				1490	2300				522
1696-13				1200	2030				544
1696-14				945	1940				545
1696-20				30.3	115				818
1696-28				*	76.3				818
1696-48				*	*				594
1696-72				*	*				494
1696-96				*	*				489
1696-120				*	*				467
1696-168				*	*				354

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1696-336				*	*				158
1696-504				*	*				65.0
1697-0									*
1697-0.25									118
1697-0.5									267
1697-1									482
1697-2									598
1697-4									789
1697-6									928
1697-8									1020
1697-12									815
1697-18									792
1697-24									722
1697-48									589
1697-72									525
1697-96									468
1697-120									438
1697-168									339
1697-336									178
1697-504									88.8
1698-0	*	*	*						*
1698-0.5	*	*	*						56.2
1698-2	*	*	*						433
1698-4	*	*	*						535
1698-8	*	*	*						635
1698-9	26.4	*	2.09						689
1698-10	42.0	*	6.55						624
1698-11	25.9	*	4.02						679
1698-12	35.4	*	8.43						639
1698-13	40.6	*	11.3						770
1698-16	43.0	*	16.1						768
1698-20	28.1	*	17.5						767
1698-24	23.5	*	20.6						616
1698-48	9.24	1.75	53.0						446

95-3.final data

3/20/97

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1698-72	12.7	7.17	200						492
1698-96	1.74	*	11.3						317
1698-120	1.21	1.17	12.7						266
1698-168	*	1.40	12.7						152
1698-336	*	1.56	5.16						46.4
1698-504	*	1.20	3.30						20.1
1699-0	*	*	*						
1699-0.25	14.5	*	*						
1699-0.5	25.8	*	2.11						
1699-1	31.0	*	6.92						
1699-2	32.4	1.01	14.9						
1699-4	30.4	2.84	20.7						
1699-6	40.8	4.08	37.9						
1699-8	31.3	4.16	36.1						
1699-12	17.1	3.68	32.0						
1699-18	10.6	2.51	23.1						
1699-24	6.77	2.01	28.3						
1699-48	3.08	5.57	60.6						
1699-72	3.68	24.1	241						
1699-96	*	4.84	29.8						
1699-120	*	1.86	11.6						
1699-168	*	5.56	27.5						
1699-336	*	1.97	6.23						
1699-504	*	6.12	14.7						
1785-0						*	*		
1785-.25						88.1	*		
1785-.5						234	4.61		
1785-1						350	14.0		
1785-2						*	*		608
1785-4						447	64.1		
1785-6						433	90.9		
1785-8						352	103		
1785-12						197	96.5		
1785-18						1570	109		
1785-24						633	109		

95-3.final data

3/20/97

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1785-48						128	78.4		
1785-72						79.1	57.4		
1785-96						54.6	35.5		
1785-120						50.3	21.6		
1785-168						40.0	10.6		
1785-336						23.5	2.81		
1785-504						15.1	*		
1786-0						*	*		*
1786-.5						*	*		127
1786-2						530	34.9		*
1786-4						2.41	*		706
1786-8						*	*		737
1786-9						1330	15.3		823
1786-10						2560	38.6		739
1786-11						2330	54.7		820
1786-12						2410	69.1		732
1786-13						1930	76.7		729
1786-16						2100	114		721
1786-20						1140	120		553
1786-24						848	131		591
1786-48						210	87.1		418
1786-96						98.4	51.1		342
1786-120						74.4	37.8		289
1786-168						56.6	22.5		257
1786-336						19.9	3.39		70.5
1786-504						13.4	2.17		23.7

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1972-H-0h				*	*				
1972-H-0.5h				3530	474				
1972-H-1h				3280	452				
1972-H-2h				3080	435				
1972-H-4h				2440	410				
1972-H-6h				1610	387				
1972-H-7h				1470	353				
1972-H-8h				862	386				
1972-H-10h				412	412				
1972-H-12h				235	395				
1972-H-18h				68.9	151				
1972-H-24h				131	*				
1972-H-48h				*	*				
1972-H-72h				*	*				
1972-H-96h				*	*				
1972-H-120h				*	*				
1972-H-168h				*	*				
1973-I-0h				*	*				*
1973-I-0.5h				12.4	*				44.8
1973-I-1h				*	*				138
1973-I-2h				*	*				332
1973-I-4h				*	*				430
1973-I-6h				*	*				430
1973-I-7h				**2500**	1630				336
1973-I-8h				**2110**	1620				275
1973-I-10h				1480	1040				282
1973-I-12h				918	999				291
1973-I-18h				59.8	252				573
1973-I-24h				41.5	122				481
1973-I-48h				*	65.7				331
1973-I-72h				*	*				368
1973-I-96h				*	*				356
1973-I-120h				*	*				222
1973-I-168h				*	*				217
1973-I-336h				*	*				68.7

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1973-I-504h				*	*				13.1
1974-I-0h				*	*				*
1974-I-0.5h				*	*				89.1
1974-I-1h				*	*				180
1974-I-2h				*	*				284
1974-I-4h				*	*				356
1974-I-6h				*	*				358
1974-I-7h				**2720**	1130				224
1974-I-8h				**1970**	916				199
1974-I-10h				1730	825				199
1974-I-12h				1230	769				209
1974-I-18h				281	178				395
1974-I-24h				45.6	85.4				430
1974-I-48h				*	*				266
1974-I-72h				*	*				278
1974-I-96h				*	*				300
1974-I-120h				*	*				162
1974-I-168h				*	*				113
1974-I-336h				*	*				22.8
1974-I-504h				*	*				5.1
1975-I-0h				*	*				*
1975-I-0.5h				*	*				163
1975-I-1h				*	*				252
1975-I-2h				*	*				486
1975-I-4h				*	*				505
1975-I-6h				*	*				518
1975-I-7h				**2660**	2410				386
1975-I-8h				**2380**	2190				378
1975-I-10h				1830	1550				375
1975-I-12h				1040	1620				352
1975-I-18h				60.4	236				591
1975-I-24h				13	64.9				611
1975-I-48h				*	*				329
1975-I-72h				*	*				356

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1975-I-96h				*	*				280
1975-I-120h				*	*				177
1975-I-168h				*	*				94.4
1975-I-336h				*	*				9.62
1975-I-504h				*	*				*
1976-I-0h				*	*				*
1976-I-0.5h				*	*				72
1976-I-1h				*	*				218
1976-I-2h				*	*				384
1976-I-4h				*	*				468
1976-I-6h				*	*				464
1976-I-7h				1890	1330				464
1976-I-8h				1980	1760				343
1976-I-10h				1550	266				370
1976-I-12h				1090	1310				326
1976-I-18h				104	1180				424
1976-I-24h				23.3	78.2				469
1976-I-48h				*	*				361
1976-I-72h				*	*				315
1976-I-96h				*	*				364
1976-I-120h				*	*				213
1976-I-168h				*	*				187
1976-I-336h				*	*				56.9
1976-I-504h				*	*				15.1
1977-H-0h				*	*				
1977-H-0.5h				2330	1280				
1977-H-1h				2370	1820				
1977-H-2h				2160	1690				
1977-H-4h				1510	1510				
1977-H-6h				1010	1320				
1977-H-7h				683	1160				
1977-H-8h				475	1040				
1977-H-10h				136	1040				
1977-H-12h				40.6	901				

bold = repeat result; NR = not run; n/a = not available

95-3.2nd preliminary data

8/26/98

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1977-H-18h				10.1	257				
1977-H-24h				*	82.6				
1977-H-48h				*	*				
1977-H-72h				*	*				
1977-H-96h				*	*				
1977-H-120h				*	*				
1977-H-168h				*	*				
1978-H-0h				*	*				
1978-H-0.5h				2350	1510				
1978-H-1h				1860	1530				
1978-H-2h				1510	1450				
1978-H-4h				1150	927				
1978-H-6h				893	1010				
1978-H-7h				784	859				
1978-H-8h				677	823				
1978-H-10h				558	629				
1978-H-12h				310	629				
1978-H-18h				26.8	171				
1978-H-24h				*	*				
1978-H-48h				*	*				
1978-H-72h				*	*				
1978-H-96h				*	*				
1978-H-120h				*	*				
1978-H-168h				*	*				
1979-F-0h						*	*		
1979-F-0.5h						14.2	*		
1979-F-1h						43.6	*		
1979-F-2h						146	3.91		
1979-F-4h						**529**	21.2		
1979-F-6h						**577**	60.4		
1979-F-8h						359	65.7		
1979-F-10h						259	75.2		
1979-F-12h						**228**	85.3		
1979-F-18h						189	83.7		

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1979-F-24h						314	67.1		
1979-F-48h						70.6	45.1		
1979-F-72h						49.9	35		
1979-F-96h						42.6	31.4		
1979-F-120h						32.6	14.3		
1979-F-168h						22.3	7.21		
1979-F-336h						8.52	*		
1979-F-504h						5.22	*		
1980-F-0h						*	*		
1980-F-0.5h						79.8	*		
1980-F-1h						240	4.43		
1980-F-2h						275	12.9		
1980-F-4h						222	24.1		
1980-F-6h						263	42.6		
1980-F-8h						200	43.1		
1980-F-10h						157	53.7		
1980-F-12h						103	47.6		
1980-F-18h						92.3	40.7		
1980-F-24h						46.5	23.7		
1980-F-48h						12.3	11.7		
1980-F-72h						7.48	5.21		
1980-F-96h						5.88	*		
1980-F-120h						7.4	*		
1980-F-168h						4.37	*		
1980-F-336h						*	*		
1980-F-504h						*	*		
1981-H-0h				*	*				
1981-H-0.5h				2460	546				
1981-H-1h				3050	809				
1981-H-2h				3020	931				
1981-H-4h				2270	750				
1981-H-6h				1840	888				
1981-H-7h				1650	697				
1981-H-8h				1520	704				

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1981-H-10h				1250	733				
1981-H-12h				937	678				
1981-H-18h				167	195				
1981-H-24h				49.3	*				
1981-H-48h				*	*				
1981-H-72h				*	*				
1981-H-96h				*	*				
1981-H-120h				*	*				
1981-H-168h				*	*				
1982-F-0h						*	*		
1982-F-0.5h						*	*		
1982-F-1h						23.9	*		
1982-F-2h						225	8.26		
1982-F-4h						219	36.3		
1982-F-6h						391	86.2		
1982-F-8h						222	84.8		
1982-F-10h						143	95.8		
1982-F-12h						102	90.9		
1982-F-18h						**873**	86		
1982-F-24h						308	105		
1982-F-48h						50.7	76.1		
1982-F-72h						23	48.9		
1982-F-96h						16.8	31.8		
1982-F-120h						14	13.3		
1982-F-168h						8.85	5.13		
1982-F-336h						3.59	*		
1982-F-504h						2.63	*		
1983-F-0h						*	*		
1983-F-0.5h						4.76	*		
1983-F-1h						11.2	*		
1983-F-2h						197	3.73		
1983-F-4h						244	16.4		
1983-F-6h						323	35.6		
1983-F-8h						272	35		

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1983-F-10h						248	39.6		
1983-F-12h						189	37.4		
1983-F-18h						**498**	40.9		
1983-F-24h						261	29.4		
1983-F-48h						82.9	21.4		
1983-F-72h						46.3	9.52		
1983-F-96h						44	5.19		
1983-F-120h						43.7	3.36		
1983-F-168h						31.9	*		
1983-F-336h						18	*		
1983-F-504h						11.7	*		
1984-I-0h				*	*				*
1984-I-0.5h				*	*				37.8
1984-I-1h				*	*				148
1984-I-2h				*	*				331
1984-I-4h				*	*				431
1984-I-6h				*	*				483
1984-I-7h				**2200**	1680				446
1984-I-8h				1860	1350				373
1984-I-10h				1130	1390				323
1984-I-12h				666	1250				307
1984-I-18h				69.2	183				451
1984-I-24h				13.4	78				468
1984-I-48h				*	*				331
1984-I-72h				*	*				366
1984-I-96h				*	*				386
1984-I-120h				*	*				238
1984-I-168h				*	*				205
1984-I-336h				*	*				80.8
1984-I-504h				*	*				**15.5**
1985-G0h						*	*		*
1985-G-0.5h						25.9	*		259
1985-G-1h						158	4.35		504
1985-G-2h						309	16		665

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1985-G-4h						**489**	38.4		689
1985-G-6h						396	63.8		779
1985-G-8h						332	76.8		779
1985-G-10h						268	94.6		**699**
1985-G-12h						260	104		**777**
1985-G-18h						**485**	94.1		**743**
1985-G-24h						**413**	85.7		562
1985-G-48h						63.6	59.8		523
1985-G-72h						29	30		418
1985-G-96h						24.9	14.9		388
1985-G-120h						24.5	7.14		334
1985-G-168h						16	3.54		219
1985-G-336h						7.72	*		57.7
1985-G-504h						5.32	*		24.8
1986-G-0h						*	*		*
1986-G-0.5h						110	*		420
1986-G-1h						220	6.17		493
1986-G-2h						311	19		673
1986-G-4h						217	31		788
1986-G-6h						198	51.3		**646**
1986-G-8h						180	53.1		**751**
1986-G-10h						109	59.5		**816**
1986-G-12h						78.7	58.5		**814**
1986-G-18h						286	59		**686**
1986-G-24h						142	46.4		593
1986-G-48h						19.7	26.1		513
1986-G-72h						12.2	14.5		389
1986-G-96h						10.3	8.74		393
1986-G-120h						9.11	4.3		306
1986-G-168h						5.76	*		212
1986-G-336h						*	*		53.7
1986-G-504h						*	*		18.4
1987-G-0h						*	*		*
1987-G-0.5h						81.9	*		211

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1987-G-1h						**437**	5.17		502
1987-G-2h						**567**	15		654
1987-G-4h						**505**	26.2		717
1987-G-6h						**337**	57		**813**
1987-G-8h						346	60.2		**690**
1987-G-10h						226	67.7		**688**
1987-G-12h						151	65		**767**
1987-G-18h						113	55		736
1987-G-24h						63.5	35.5		579
1987-G-48h						29.7	29.8		574
1987-G-72h						19.8	16.3		413
1987-G-96h						16.5	10.4		429
1987-G-120h						13.1	3.84		292
1987-G-168h						8.15	*		190
1987-G-336h						3.43	*		28
1987-G-504h						*	*		7.32
1988-H-0h				*	57.6				
1988-H-0.5h				1840	1220				
1988-H-1h				1860	1470				
1988-H-2h				1450	1300				
1988-H-4h				954	1070				
1988-H-6h				593	931				
1988-H-7h				392	797				
1988-H-8h				259	844				
1988-H-10h				78.4	738				
1988-H-12h				26.8	728				
1988-H-18h				*	231				
1988-H-24h				*	171				
1988-H-48h				*	67.1				
1988-H-72h				*	*				
1988-H-96h				*	*				
1988-H-120h				*	*				
1988-H-168h				*	*				
1989-G-0h						*	*		*

bold = repeat result; NR = not run; n/a = not available

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	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1989-G-0.5h						2.3	*		13
1989-G-1h						71.3	*		144
1989-G-2h(100ul)						114	11.4		**237**
1989-G-4h						138	17.5		378
1989-G-6h						318	61.9		715
1989-G-8h						**468**	64.6		**773**
1989-G-10h						**372**	97.4		**846**
1989-G-12h						317	91.1		n/a
1989-G-18h						**582**	90.4		712
1989-G-24h						232	69.8		564
1989-G-48h						57.2	43.3		416
1989-G-72h						31.4	21.5		355
1989-G-96h						27.5	15.9		358
1989-G-120h						21.5	6.95		261
1989-G-168h						14.1	3.95		180
1989-G-336h						5.06	*		33.4
1989-G-504h						*	*		4.96
1990-G-0h						*	*		*
1990-G-0.5h						18.7	*		76.4
1990-G-1h						128	3.91		275
1990-G-2h						175	10.7		534
1990-G-4h						282	28.9		763
1990-G-6h						376	55.9		**771**
1990-G-8h						288	60		**772**
1990-G-10h						188	68.3		**829**
1990-G-12h						147	70.1		**864**
1990-G-18h						n/a	n/a		n/a
1990-G-24h						213	54.8		626
1990-G-48h						49.4	33.5		602
1990-G-72h						33.4	17.8		446
1990-G-96h						29.7	12.3		494
1990-G-120h						22.4	5.99		387
1990-G-168h						15.6	3.94		265
1990-G-336h						6.79	*		80.8
1990-G-504h						4.15	*		31.5

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1991-I-0h				*	*				*
1991-I-0.5h				*	*				111
1991-I-1h				*	*				250
1991-I-2h				*	*				382
1991-I-4h				*	*				516
1991-I-6h				*	*				502
1991-I-7h				**3380**	891				365
1991-I-8h				**3350**	1110				287
1991-I-10h				**2490**	1080				224
1991-I-12h				1560	1240				237
1991-I-18h				108	239				628
1991-I-24h				32.2	76.5				619
1991-I-48h				*	*				379
1991-I-72h				*	*				373
1991-I-96h				*	*				393
1991-I-120h				*	*				232
1991-I-168h				*	*				181
1991-I-336h				*	*				62.2
1991-I-504h				NR	*				NR
1992-H-0h				*	*				
1992-H-0.5h				1470	815				
1992-H-1h				2430	1940				
1992-H-2h				1980	1970				
1992-H-4h				1650	1600				
1992-H-6h				1270	1400				
1992-H-7h				1130	1350				
1992-H-8h				898	1140				
1992-H-10h				590	1180				
1992-H-12h				272	972				
1992-H-18h				29.2	253				
1992-H-24h				13.8	169				
1992-H-48h				*	64.9				
1992-H-72h				*	*				
1992-H-96h				*	*				

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1992-H-120h				*	54.2				
1992-H-168h				*	*				
1993-F-0h						*	*		
1993-F-0.5h						195	3.05		
1993-F-1h						**695**	12.7		
1993-F-2h						**580**	23.4		
1993-F-4h						374	51.6		
1993-F-6h						373	87.3		
1993-F-8h						255	86.3		
1993-F-10h						170	93.9		
1993-F-12h						136	92.6		
1993-F-18h						**792**	90.2		
1993-F-24h						21	9.86		
1993-F-48h						379	85.9		
1993-F-72h						61.9	54.1		
1993-F-96h						31.7	30		
1993-F-120h						23.9	18.7		
1993-F-168h						11.9	3.84		
1993-F-336h						5.07	*		
1993-F-504h						*	*		
1994-F-0h						*	*		
1994-F-0.5h						47.5	*		
1994-F-1h						201	5.35		
1994-F-2h						313	25.3		
1994-F-4h						**449**	60.4		
1994-F-6h						**948**	163		
1994-F-8h						**887**	155		
1994-F-10h						**754**	200		
1994-F-12h						**641**	182		
1994-F-18h						287	140		
1994-F-24h						175	104		
1994-F-48h						56	77.6		
1994-F-72h						37.5	53.7		
1994-F-96h						**15.3**	**28**		

bold = repeat result; NR = not run; n/a = not available

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	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
1994-F-120h						25.3	18.5		
1994-F-168h						14	7.68		
1994-F-336h						5.97	*		
1994-F-504h						2.17	*		
1995-G-0h						*	*		*
1995-G-0.5h						53.1	*		160
1995-G-1h						173	5.02		305
1995-G-2h						238	15.6		587
1995-G-4h						313	32.9		651
1995-G-6h						**569**	78.6		**859**
1995-G-8h						324	82		**821**
1995-G-10h						227	95.2		**776**
1995-G-12h						175	87.3		**803**
1995-G-18h						**573**	115		810
1995-G-24h						**1180**	91		590
1995-G-48h						208	63.3		587
1995-G-72h						113	38.3		518
1995-G-96h						89.2	30.8		517
1995-G-120h						79.8	16.1		389
1995-G-168h						47.9	11.2		343
1995-G-336h						27.3	3.27		76.9
1995-G-504h						12.2	*		12.4
CM2006-C-0h								*	*
CM2006-C-0.5h								*	336
CM2006-C-1h								*	526
CM2006-C-2h								*	610
CM2006-C-4h								878	724
CM2006-C-6h								n/a	665
CM2006-C-8h								n/a	602
CM2006-C-10h								n/a	770
CM2006-C-12h								n/a	n/a
CM2006-C-18h								861	n/a
CM2006-C-24h								547	541
CM2006-C-48h								254	392

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
CM2006-C-72h								91.9	337
CM2006-C-96h								42.9	246
CM2006-C-120h								19.5	222
CM2006-C-168h								*	127
CM2006-C-336h								*	15.8
CM2006-C-504h								*	*
CM2007-C-0h								*	*
CM2007-C-0.5h								*	141
CM2007-C-1h								*	674
CM2007-C-2h								*	n/a
CM2007-C-4h								929	n/a
CM2007-C-6h								n/a	758
CM2007-C-8h								n/a	n/a
CM2007-C-10h								n/a	n/a
CM2007-C-12h								n/a	n/a
CM2007-C-18h								994	782
CM2007-C-24h								878	709
CM2007-C-48h								393	523
CM2007-C-72h								244	542
CM2007-C-96h								122	371
CM2007-C-120h								85.2	457
CM2007-C-168h								28.4	315
CM2007-C-336h								*	165
CM2007-C-504h								*	98.8
BM2008-B-0h								*	
BM2008-B-0.5h								9.96	
BM2008-B-1h								184	
BM2008-B-2h								464	
BM2008-B-4h								738	
BM2008-B-6h								793	
BM2008-B-8h								666	
BM2008-B-10h								572	
BM2008-B-12h								594	
BM2008-B-18h								363	

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
BM2008-B-24h								234	
BM2008-B-48h								87.5	
BM2008-B-72h								32.3	
BM2008-B-96h								8.62	
BM2008-B-120h								*	
BM2008-B-168h								*	
BM2008-B-336h								*	
BM2008-B-504h								*	
BM2009-B-0h								*	
BM2009-B-0.5h								*	
BM2009-B-1h								22.8	
BM2009-B-2h								338	
BM2009-B-4h								579	
BM2009-B-6h								630	
BM2009-B-8h								639	
BM2009-B-10h								641	
BM2009-B-12h								599	
BM2009-B-18h								309	
BM2009-B-24h								241	
BM2009-B-48h								103	
BM2009-B-72h								39.7	
BM2009-B-96h								12.8	
BM2009-B-120h								*	
BM2009-B-168h								*	
BM2009-B-336h								*	
BM2009-B-504h								*	
AM2010-A-0h									*
AM2010-A-0.5h									116
AM2010-A-1h									354
AM2010-A-2h									517
AM2010-A-4h									526
AM2010-A-6h									651
AM2010-A-8h									703
AM2010-A-10h									695

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
AM2010-A-12h									638
AM2010-A-18h									580
AM2010-A-24h									372
AM2010-A-48h									410
AM2010-A-72h									433
AM2010-A-96h									393
AM2010-A-120h									343
AM2010-A-168h									255
AM2010-A-336h									150
AM2010-A-504h									79.3
AM2011-A-0h									*
AM2011-A-0.5h									311
AM2011-A-1h									605
AM2011-A-2h									759
AM2011-A-4h									**704**
AM2011-A-6h									**1000**
AM2011-A-8h									**998**
AM2011-A-10h									**982**
AM2011-A-12h									**924**
AM2011-A-18h									**822**
AM2011-A-24h									n/a
AM2011-A-48h									590
AM2011-A-72h									587
AM2011-A-96h									499
AM2011-A-120h									346
AM2011-A-168h									283
AM2011-A-336h									89.9
AM2011-A-504h									33.4
CM2012-C-0h								*	n/a
CM2012-C-0.5h								*	**26**
CM2012-C-1h								*	375
CM2012-C-2h								*	603
CM2012-C-4h								n/a	**1070**
CM2012-C-6h								n/a	**895**

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
CM2012-C-8h								n/a	**886**
CM2012-C-10h								n/a	**983**
CM2012-C-12h								n/a	**935**
CM2012-C-18h								n/a	**881**
CM2012-C-24h								n/a	n/a
CM2012-C-48h								745	571
CM2012-C-72h								325	538
CM2012-C-96h								148	434
CM2012-C-120h								59.5	418
CM2012-C-168h								17.1	342
CM2012-C-336h								*	96.8
CM2012-C-504h								*	28.5
AM2013-A-0h									*
AM2013-A-0.5h									80.1
AM2013-A-1h									428
AM2013-A-2h									**674**
AM2013-A-4h									**923**
AM2013-A-6h									**861**
AM2013-A-8h									**979**
AM2013-A-10h									n/a
AM2013-A-12h									n/a
AM2013-A-18h									n/a
AM2013-A-24h									728
AM2013-A-48h									602
AM2013-A-72h									628
AM2013-A-96h									444
AM2013-A-120h									314
AM2013-A-168h									187
AM2013-A-336h									38.9
AM2013-A-504h									14.6
BM2014-B-0h								122	
BM2014-B-0.5h								152	
BM2014-B-1h								635	
BM2014-B-2h								**1060**	

bold = repeat result; NR = not run; n/a = not available

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	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
BM2014-B-4h								**1200**	
BM2014-B-6h								**1130**	
BM2014-B-8h								**1000**	
BM2014-B-10h								**902**	
BM2014-B-12h								**1040**	
BM2014-B-18h								**1180**	
BM2014-B-24h								**1290**	
BM2014-B-48h								805	
BM2014-B-72h								528	
BM2014-B-96h								224	
BM2014-B-120h								101	
BM2014-B-168h								38.7	
BM2014-B-336h								*	
BM2014-B-504h								*	
EM2015-E-0h	*	*	*						*
EM2015-E-0.5h	8.78	*	*						79.9
EM2015-E-1h	90.9	*	11.1						361
EM2015-E-2h	56.9	*	19						541
EM2015-E-4h	138	7.04	125						565
EM2015-E-6h	69.6	6.75	70.6						719
EM2015-E-8h	127	17.5	256						776
EM2015-E-10h	92.2	18.9	233						**764**
EM2015-E-12h	54.9	13.5	145						**706**
EM2015-E-18h	55.9	25	324						**754**
EM2015-E-24h	45.3	37.8	500						604
EM2015-E-48h	19.1	33.6	420						472
EM2015-E-72h	8.01	36.6	360						436
EM2015-E-96h	*	25.1	202						291
EM2015-E-120h	*	12.9	96.1						251
EM2015-E-168h	*	35.8	259						209
EM2015-E-336h	*	12.7	48.2						72.6
EM2015-E-504h	*	8.36	21.2						24.5
AM2017-A-0h									*
AM2017-A-0.5h									73.1

bold = repeat result; NR = not run; n/a = not available

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	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
AM2017-A-1h									130
AM2017-A-2h									183
AM2017-A-4h									305
AM2017-A-6h									373
AM2017-A-8h									370
AM2017-A-10h									443
AM2017-A-12h									338
AM2017-A-18h									343
AM2017-A-24h									249
AM2017-A-48h									157
AM2017-A-72h									145
AM2017-A-96h									114
AM2017-A-120h									67.3
AM2017-A-168h									41.9
AM2017-A-336h									5.05
AM2017-A-504h									*
DM2018-D-0	*	*	*						
DM2018-D-0.5	37.8	*	*						
DM2018-D-1	77	*	11.3						
DM2018-D-2	75.9	*	28.9						
DM2018-D-4	116	*	98.3						
DM2018-D-6	85.7	6.5	117						
DM2018-D-8	151	16.6	276						
DM2018-D-10	78.9	15.5	194						
DM2018-D-12	85.2	25.4	345						
DM2018-D-18	75.8	34	465						
DM2018-D-24	46.9	42.7	519						
DM2018-D-48	13.4	30.9	344						
DM2018-D-72	6.85	31.1	287						
DM2018-D-96	*	20.9	169						
DM2018-D-120	*	14.3	130						
DM2018-D-168	*	12.2	91						
DM2018-D-336	*	11.2	58.4						
DM2018-D-504	*	7.38	32						

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
AM2019-A-0h									*
AM2019-A-0.5h									112
AM2019-A-1h									213
AM2019-A-2h									303
AM2019-A-4h									413
AM2019-A-6h									526
AM2019-A-8h									646
AM2019-A-10h									608
AM2019-A-12h									528
AM2019-A-18h									436
AM2019-A-24h									309
AM2019-A-48h									234
AM2019-A-72h									305
AM2019-A-96h									263
AM2019-A-120h									158
AM2019-A-168h									143
AM2019-A-336h									51.6
AM2019-A-504h									20
CM2020-C-0h								*	*
CM2020-C-0.5h								*	130
CM2020-C-1h								*	332
CM2020-C-2h								*	499
CM2020-C-4h								586	566
CM2020-C-6h								896	609
CM2020-C-8h								821	579
CM2020-C-10h								878	658
CM2020-C-12h								638	637
CM2020-C-18h								876	789
CM2020-C-24h								473	454
CM2020-C-48h								289	429
CM2020-C-72h								215	487
CM2020-C-96h								97.3	372
CM2020-C-120h								57	350
CM2020-C-168h								20.5	279
CM2020-C-336h								*	133

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
CM2020-C-504h								*	70.5
DM2021-D-0	*	*	*						
DM2021-D-0.5	65.2	*	*						
DM2021-D-1	81.6	*	6.97						
DM2021-D-2	125	*	19						
DM2021-D-4	250	*	89.1						
DM2021-D-6	102	*	46.7						
DM2021-D-8	216	*	153						
DM2021-D-10	118	*	102						
DM2021-D-12	122	4.33	138						
DM2021-D-18	166	15.8	426						
DM2021-D-24	122	17.8	427						
DM2021-D-48	27.7	13.8	280						
DM2021-D-72	19	19.4	361						
DM2021-D-96	10.1	18.2	279						
DM2021-D-120	5.46	11.9	181						
DM2021-D-168	*	12	139						
DM2021-D-336	*	7.51	52.1						
DM2021-D-504	*	4.96	30.5						
DM2022-D-0	*	*	*						
DM2022-D-0.5	59.7	*	4.81						
DM2022-D-1	42.7	*	6.88						
DM2022-D-2	65.8	*	18.7						
DM2022-D-4	116	*	68						
DM2022-D-6	60.9	*	43.5						
DM2022-D-8	138	7.55	168						
DM2022-D-10	126	12.6	240						
DM2022-D-12	121	18.1	347						
DM2022-D-18	58.6	19.8	300						
DM2022-D-24	67.6	32.1	521						
DM2022-D-48	8.29	10	133						
DM2022-D-72	4.18	10.5	111						
DM2022-D-96	*	14.3	95.4						
DM2022-D-120	*	*	27.6						

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
DM2022-D-168	*	4.91	34.3						
DM2022-D-336	*	*	15.1						
DM2022-D-504	*	*	10.7						
BM2023-B-0h								*	
BM2023-B-0.5h								55.8	
BM2023-B-1h								503	
BM2023-B-2h								996	
BM2023-B-4h								**1210**	
BM2023-B-6h								967	
BM2023-B-8h								**860**	
BM2023-B-10h								759	
BM2023-B-12h								832	
BM2023-B-18h								643	
BM2023-B-24h								412	
BM2023-B-48h								177	
BM2023-B-72h								63.1	
BM2023-B-96h								32.1	
BM2023-B-120h								13	
BM2023-B-168h								*	
BM2023-B-336h								*	
BM2023-B-504h								*	
DM2024-D-0	*	*	*						
DM2024-D-0.5	40.3	*	*						
DM2024-D-1	53.2	*	6.3						
DM2024-D-2	92.1	*	28						
DM2024-D-4	90.8	*	68.8						
DM2024-D-6	38.5	*	57						
DM2024-D-8	76.6	4.86	177						
DM2024-D-10	47	5.59	146						
DM2024-D-12	63.3	14.9	347						
DM2024-D-18	37.3	15.2	358						
DM2024-D-24	21.7	14.3	309						
DM2024-D-48	5.51	11	179						
DM2024-D-72	*	10.5	120						

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
DM2024-D-96	*	8.83	86.9						
DM2024-D-120	*	4.33	42.2						
DM2024-D-168	*	8.51	61.9						
DM2024-D-336	*	8.03	39.7						
DM2024-D-504	*	4.07	18.6						
EM2025-E-0h	*	*	*						*
EM2025-E-0.5h	67.6	*	*						223
EM2025-E-1h	105	*	9.63						755
EM2025-E-2h	163	*	40.5						776
EM2025-E-4h	214	*	119						932
EM2025-E-6h	77.3	*	43.9						1050
EM2025-E-8h	190	9.78	226						1060
EM2025-E-10h	79.4	6.49	117						n/a
EM2025-E-12h	92.8	23	443						n/a
EM2025-E-18h	61.8	7.63	104						n/a
EM2025-E-24h	62.8	28	477						n/a
EM2025-E-48h	18.3	21	305						691
EM2025-E-72h	11.9	28.8	332						657
EM2025-E-96h	4.74	18.1	167						493
EM2025-E-120h	*	12	92.2						450
EM2025-E-168h	*	7.55	53.6						370
EM2025-E-336h	*	13.2	64.8						125
EM2025-E-504h	*	7.68	29.9						37.3
BM2026-B-0h								*	
BM2026-B-0.5h								*	
BM2026-B-1h								*	
BM2026-B-2h								327	
BM2026-B-4h								**770**	
BM2026-B-6h								**914**	
BM2026-B-8h								**969**	
BM2026-B-10h								**981**	
BM2026-B-12h								**864**	
BM2026-B-18h								912	
BM2026-B-24h								711	

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	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
BM2026-B-48h								453	
BM2026-B-72h								299	
BM2026-B-96h								166	
BM2026-B-120h								119	
BM2026-B-168h								44.4	
BM2026-B-336h								*	
BM2026-B-504h								*	
DM2027-D-0	*	*	*						
DM2027-D-0.5	62.5	*	5.54						
DM2027-D-1	78.5	*	14.1						
DM2027-D-2	91.5	*	29.4						
DM2027-D-4	84.4	*	63.4						
DM2027-D-6	50.9	*	49.4						
DM2027-D-8	120	11	218						
DM2027-D-10	59.2	10.2	139						
DM2027-D-12	42	6.56	91.5						
DM2027-D-18	60.2	21.2	350						
DM2027-D-24	48	33.6	492						
DM2027-D-48	7.28	14.5	186						
DM2027-D-72	4.19	19.9	218						
DM2027-D-96	*	16.1	144						
DM2027-D-120	*	5.89	60.3						
DM2027-D-168	*	7.76	57.1						
DM2027-D-336	*	5.4	22.5						
DM2027-D-504	*	*	8.27						
EM2028-E-0h	*	*	*						*
EM2028-E-0.5h	47.8	*	*						307
EM2028-E-1h	92.4	*	9.26						792
EM2028-E-2h	146	*	29.8						n/a
EM2028-E-4h	166	*	82						n/a
EM2028-E-6h	89.1	*	41.2						n/a
EM2028-E-8h	103	5	102						n/a
EM2028-E-10h	108	4.78	104						n/a
EM2028-E-12h	44.3	*	41.2						**1060**

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
EM2028-E-18h	54.1	8.27	176						**895**
EM2028-E-24h	12.9	8.62	122						641
EM2028-E-48h	49.6	13.3	256						n/a
EM2028-E-72h	6.64	8.6	107						533
EM2028-E-96h	4.12	6.36	74.9						418
EM2028-E-120h	*	8.88	92						338
EM2028-E-168h	*	10	75.1						296
EM2028-E-336h	*	6.42	44.8						168
EM2028-E-504h	*	*	13.7						57.8
CM2029-C-0h								*	*
CM2029-C-0.5h								*	91.1
CM2029-C-1h								*	175
CM2029-C-2h								*	306
CM2029-C-4h								698	413
CM2029-C-6h								**968**	405
CM2029-C-8h								**1100**	380
CM2029-C-10h								**1100**	428
CM2029-C-12h								**966**	464
CM2029-C-18h								**867**	423
CM2029-C-24h								669	294
CM2029-C-48h								375	250
CM2029-C-72h								267	250
CM2029-C-96h								143	209
CM2029-C-120h								107	238
CM2029-C-168h								41.9	160
CM2029-C-336h								*	61.3
CM2029-C-504h								*	26.9
DM2030-D-0	*	*	*						
DM2030-D-0.5	95.8	*	6.45						
DM2030-D-1	88.4	*	9.96						
DM2030-D-2	130	*	27.7						
DM2030-D-4	142	*	63.7						
DM2030-D-6	69.9	*	44.1						
DM2030-D-8	207	5.08	181						

bold = repeat result; NR = not run; n/a = not available

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	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
DM2030-D-10	89.6	*	97.1						
DM2030-D-12	122	10.3	232						
DM2030-D-18	132	11.3	324						
DM2030-D-24	71.9	15.2	311						
DM2030-D-48	18.6	9.34	191						
DM2030-D-72	10.3	16.4	255						
DM2030-D-96	*	8.61	120						
DM2030-D-120	*	4.64	75						
DM2030-D-168	*	9.4	83.3						
DM2030-D-336	*	8.11	50.1						
DM2030-D-504	*	*	17.4						
AM2031-A-0h									*
AM2031-A-0.5h									56.6
AM2031-A-1h									220
AM2031-A-2h									265
AM2031-A-4h									390
AM2031-A-6h									584
AM2031-A-8h									681
AM2031-A-10h									689
AM2031-A-12h									676
AM2031-A-18h									620
AM2031-A-24h									562
AM2031-A-48h									398
AM2031-A-72h									374
AM2031-A-96h									271
AM2031-A-120h									211
AM2031-A-168h									156
AM2031-A-336h									34.7
AM2031-A-504h									11.2
CM2032-C-0h								*	*
CM2032-C-0.5h								*	26.7
CM2032-C-1h								*	n/a
CM2032-C-2h								*	n/a
CM2032-C-4h								85.8	n/a

bold = repeat result; NR = not run; n/a = not available

	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
CM2032-C-6h								877	n/a
CM2032-C-8h								911	n/a
CM2032-C-10h								**988**	n/a
CM2032-C-12h								938	n/a
CM2032-C-18h								787	n/a
CM2032-C-24h								**1090**	**706**
CM2032-C-48h								629	609
CM2032-C-72h								246	578
CM2032-C-96h								81.4	368
CM2032-C-120h								48	459
CM2032-C-168h								8.88	349
CM2032-C-336h								*	102
CM2032-C-504h								*	31
EM2033-E-0h	*	*	*						*
EM2033-E-0.5h	91.6	*	*						157
EM2033-E-1h	83.2	*	12.7						616
EM2033-E-2h	76.6	*	26.5						669
EM2033-E-4h	143	*	116						**637**
EM2033-E-6h	123	5.35	188						**743**
EM2033-E-8h	139	9.14	286						**750**
EM2033-E-10h	47.6	*	68.3						**925**
EM2033-E-12h	43.7	4.12	67.2						**828**
EM2033-E-18h	46.2	14.1	358						**676**
EM2033-E-24h	26.2	14.7	321						701
EM2033-E-48h	6.55	12.4	195						454
EM2033-E-72h	4.3	13.5	170						349
EM2033-E-96h	*	12.7	131						248
EM2033-E-120h	*	11.7	117						218
EM2033-E-168h	*	13.3	132						153
EM2033-E-336h	*	7.27	40.4						33.2
EM2033-E-504h	*	*	17.2						8.41
EM2034-E-0h	*	*	*						*
EM2034-E-0.5h	**55.8**	*	*						77.4
EM2034-E-1h	**152**	*	**14.3**						668

bold = repeat result; NR = not run; n/a = not available

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	Chloroquine (ng/ml)	Di chloro (ng/ml)	Mono choloro (ng/ml)	Quinine (ng/ml)	Doxy (ng/ml)	Halofantrine (ng/ml)	Halo metab (ng/ml)	Mefloquine (ng/ml)	WR 238605 (ng/ml)
EM2034-E-2h	**148**	*	**38.9**						**838**
EM2034-E-4h	**210**	*	**139**						**944**
EM2034-E-6h	**134**	**5.74**	**156**						**1180**
EM2034-E-8h	**182**	**13.4**	**274**						**1100**
EM2034-E-10h	**176**	*	**116**						**1420**
EM2034-E-12h	**105**	**19.6**	**319**						**1330**
EM2034-E-18h	**111**	**55.9**	**797**						**1320**
EM2034-E-24h	**63.7**	**56.6**	**752**						**895**
EM2034-E-48h	**19.4**	**43.7**	**501**						**590**
EM2034-E-72h	**9.12**	**58**	**522**						649
EM2034-E-96h	*	**26.8**	**280**						490
EM2034-E-120h	*	**31.6**	**335**						425
EM2034-E-168h	*	**19.4**	**151**						340
EM2034-E-336h	*	**7.23**	**44.4**						151
EM2034-E-504h	*	**4.39**	**33.2**						41.5
EM2035-E-0h	*	*	*						*
EM2035-E-0.5h	12.1	*	*						74.9
EM2035-E-1h	78.4	*	*						447
EM2035-E-2h	156	*	19.3						**721**
EM2035-E-4h	186	*	53.8						**895**
EM2035-E-6h	164	*	75.8						**923**
EM2035-E-8h	214	*	130						**1190**
EM2035-E-10h	212	18.5	447						n/a
EM2035-E-12h	121	*	106						**1450**
EM2035-E-18h	159	10.1	362						**1070**
EM2035-E-24h	142	12.2	467						**1050**
EM2035-E-48h	38.8	6.87	218						**626**
EM2035-E-72h	21.4	14.5	324						682
EM2035-E-96h	10.4	13.8	254						588
EM2035-E-120h	4.84	9.25	145						449
EM2035-E-168h	*	10.8	168						390
EM2035-E-336h	*	4.71	46.5						162
EM2035-E-504h	*	4.59	36.9						51.2

bold = repeat result; NR = not run; n/a = not available

Hal/P 95-4 Final Data 11/24/97

HALOFANTRINE					
Subject	17	18	19	20	21
	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)
1-0	*	*	5.71	1.36	*
1-0.5	*	*	14.8	5.55	*
1-1	*	8.61	101	11.3	*
1-2	*	54.9	85.1	24.2	*
1-3	*	185	145	40.2	*
1-4	*	308	167	51.5	*
1-6	*	267	193	42.0	*
1-8	NS	129	113	31.0	*
1-10	NS	158	78.0	23.2	*
1-12	NS	152	103	18.1	*
2-0	NS	68.5	54.0	9.72	*
3-0	NS	135	171	20.3	*
4-0	NS	155	90.3	35.3	*
4-2	NS	148	140	47.1	*
4-4	NS	288	298	91.2	*
4-6	NS	290	350	*	*
4-8	NS	241	254	78.0	*
4-12	NS	161	162	61.7	*
5-0	NS	112	156	33.6	*
6-0	NS	123	137	44.4	*
7-0	NS	182	105	46.4	*
7-2	NS	195	365	62.0	*
7-4	NS	419	335	114	*
7-6	NS	468	373	94.4	*
7-8	NS	299	323	96.0	*
7-12	NS	282	178	62.8	*
8-0	NS	158	154	41.0	*
9-0	NS	149	123	42.4	*
10-0	NS	160	134	53.4	*
11-0	NS	182	158	52.1	*
12-0	NS	192	182	55.4	*
13-0	NS	155	154	62.4	*
14-0	NS	176	383	46.3	*
14-2	NS	191	NS	63.6	*
14-4	NS	380	NS	100	*
14-6	NS	395	NS	93.9	*
14-8	NS	294	NS	89.8	*
14-12	NS	244	NS	84.4	*
15-0	NS	157	205	57.2	*
15-2	NS	NS	342	NS	NS
15-4	NS	NS	410	NS	NS
15-6	NS	NS	422	NS	NS
15-8	NS	NS	335	NS	NS

* = below assay sensitivity (1 ng/ml); NS = no sample

Hal/P 95-4 Final Data 11/24/97

HALOFANTRINE					
Subject	17	18	19	20	21
15-12	NS	NS	253	NS	NS
16-0	NS	209	179	60.9	*
17-0	NS	168	204	59.9	*
18-0	NS	165	131	46.7	*
19-0	NS	148	153	57.8	*
20-0	NS	143	191	71.4	*
21-0	NS	160	174	67.2	*
21-2	NS	269	204	84.3	*
21-4	NS	306	339	177	*
21-6	NS	392	290	112	*
21-8	NS	269	352	110	*
21-12	NS	214	187	101	*
22-0	NS	158	153	66.8	*
25-0	NS	136	118	41.2	*
29-0	NS	151	131	161	*
30-0	NS	NS	NS	166	NS
32-0	NS	391	152	97.9	*
33-0	NS	NS	131	83.9	*
36-0	NS	NS	141	(DAY 35)84.9	*
39-0	NS	NS	125	57.0	*
42-0	NS	467	132	68.8	*
42-0.5	NS	543	109	60.3	*
42-1	NS	483	147	88.3	*
42-2	NS	534	156	82.5	*
42-3	NS	611	258	188	*
42-4	NS	826	278	150	*
42-6	NS	168	348	168	*
42-8	NS	150	292	152	*
42-10	NS	137	225	123	*
42-12	NS	123	263	122	*
43	NS	83.3	182	80.7	*
44	NS	NS	145	42.3	*
45	NS	57.6	87.2	37.3	*
48	NS	20.7	54.8	23.5	*
51	NS	(DAY 52)25.2	62.3	22.3	*
54	NS	20.4	45.4	NS	*
57	NS	117	49.5	(DAY 60)16	*
72	NS	65.6	33.1	2.20	*
180	NS	NS	5.54	2.38	NS
	0	68	76	73	0

* = below assay sensitivity (1 ng/ml); NS = no sample

Hal/P 95-4 Final Data 11/24/97

	METABOLITE				
	Subject	17	18	19	20
	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)	(ng/ml)
	1-0	*	*	*	*
	1-0.5	*	*	*	*
	1-1	*	*	6.55	2.44
	1-2	*	7.21	18.2	7.25
	1-3	*	24.9	29.1	16.2
	1-4	*	36.2	42.2	23.7
	1-6	*	35.6	50.6	34.1
	1-8	NS	43.8	39.9	36.4
	1-10	NS	41.7	47.4	35.2
	1-12	NS	42.5	47.3	36.6
	2-0	NS	39.0	43.4	24.9
	3-0	NS	135	115	57.0
	4-0	NS	178	134	114
	4-2	NS	166	145	114
	4-4	NS	202	170	155
	4-6	NS	163	170	*
	4-8	NS	137	154	146
	4-12	NS	123	156	124
	5-0	NS	180	162	111
	6-0	NS	208	223	141
	7-0	NS	225	224	147
	7-2	NS	238	262	158
	7-4	NS	236	258	177
	7-6	NS	271	302	176
	7-8	NS	250	201	166
	7-12	NS	254	206	138
	8-0	NS	243	300	159
	9-0	NS	259	288	178
	10-0	NS	299	267	184
	11-0	NS	331	321	199
	12-0	NS	336	399	182
	13-0	NS	321	378	217
	14-0	NS	370	469	175
	14-2	NS	337	NS	192
	14-4	NS	374	NS	218
	14-6	NS	336	NS	226
	14-8	NS	303	NS	201
	14-12	NS	289	NS	225
	15-0	NS	276	499	171
	15-2	NS	NS	669	NS
	15-4	NS	NS	607	NS
	15-6	NS	NS	649	NS
	15-8	NS	NS	579	NS

* = below assay sensitivity (1 ng/ml); NS = no sample

Hal/P 95-4 Final Data 11/24/97

	METABOLITE				
	Subject	17	18	19	20
	15-12	NS	NS	449	NS
	16-0	NS	336	579	196
	17-0	NS	289	584	229
	18-0	NS	321	358	180
	19-0	NS	279	491	174
	20-0	NS	304	487	201
	21-0	NS	316	471	193
	21-2	NS	466	500	206
	21-4	NS	355	460	252
	21-6	NS	365	435	258
	21-8	NS	350	620	243
	21-12	NS	315	426	196
	22-0	NS	318	498	160
	25-0	NS	306	506	236
	29-0	NS	233	468	347
	30-0	NS	NS	NS	450
	32-0	NS	257	434	524
	33-0	NS	NS	412	495
	36-0	NS	NS	355	(DAY 35)489
	39-0	NS	NS	404	456
	42-0	NS	550	302	374
	42-0.5	NS	616	294	382
	42-1	NS	581	388	462
	42-2	NS	628	348	412
	42-3	NS	633	461	519
	42-4	NS	733	450	523
	42-6	NS	132	473	502
	42-8	NS	119	443	525
	42-10	NS	112	431	523
	42-12	NS	132	450	505
	43	NS	111	313	484
	44	NS	NS	490	356
	45	NS	92.8	370	348
	48	NS	77.6	223	188
	51	NS	(DAY 52)76.2	182	126
	54	NS	47.0	63.5	NS
	57	NS	153	42.8	(DAY 60)44.4
	72	NS	67.7	14.4	1.91
	180	NS	NS	2.43	1.64
	0	0	67	74	71

* = below assay sensitivity (1 ng/ml); NS = no sample

Final Data Hal/P 95-4 Chiral

5/4/98

001 (JKS)		002 (BSH)		04 (WPS)		005 (SGA)		007 (DAN)		008 (AYB)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	11.4	D 1-H 1	*	D 1-H 1	15.9
D 1-H 2	*	D 1-H 2	*	D 1-H 2	16.4	D 1-H 2	19.9	D 1-H 2	10.8	D 1-H 2	37.6
D 1-H 3	*	D 1-H 3	*	D 1-H 3	52.6	D 1-H 3	19.5	D 1-H 3	14.9	D 1-H 3	65.3
D 1-H 4	10.9	D 1-H 4	*	D 1-H 4	35.1	D 1-H 4	15.1	D 1-H 4	28.4	D 1-H 4	78.9
D 1-H 6	15.1	D 1-H 6	31.5	D 1-H 6	71.1	D 1-H 6	34.7	D 1-H 6	23.3	D 1-H 6	162
D 1-H 8	15.0	D 1-H 8	25.0	D 1-H 8	44.5	D 1-H 8	20.3	D 1-H 8	23.5	D 1-H 8	102
D 1-H 10	17.2	D 1-H 10	29.5	D 1-H 10	18.9	D 1-H 10	37.4	D 1-H 10	20.9	D 1-H 10	61.9
D 1-H 12	12.9	D 1-H 12	20.1	D 1-H 12	29.6	D 1-H 12	14.5	D 1-H 12	17.5	D 1-H 12	62.1
D 2-H 0	*	D 2-H 0	13.0	D 2-H 0	14.6	D 2-H 0	*	D 2-H 0	10.3	D 2-H 0	24.1
D 3-H 0	10.9	D 3-H 0	*	D 3-H 0	25.6	D 3-H 0	14.3	D 3-H 0	50.8	D 3-H 0	45.5
D 4-H 0	32.0	D 4-H 0	30.4	D 4-H 0	79.9	D 4-H 0	20.1	D 4-H 0	75.4	D 4-H 0	33.2
D 4-H 2	30.6	D 4-H 2	37.7	D 4-H 2	308	D 4-H 2	22.2	D 4-H 2	342	D 4-H 2	67.3
D 4-H 4	73.2	D 4-H 4	79.1	D 4-H 4	348	D 4-H 4	78.6	D 4-H 4	572	D 4-H 4	152
D 4-H 6	47.9	D 4-H 6	63.5	D 4-H 6	446	D 4-H 6	50.2	D 4-H 6	456	D 4-H 6	114
D 4-H 8	38.9	D 4-H 8	46.4	D 4-H 8	292	D 4-H 8	67.3	D 4-H 8	290	D 4-H 8	103
D 4-H 12	54.6	D 4-H 12	33.1	D 4-H 12	246	D 4-H 10	52.6	D 4-H 12	235	D 4-H 12	136
D 5-H 0	25.9	D 5-H 0	26.9	D 5-H 0	107	D 5-H 0	26.7	D 5-H 0	88.4	D 5-H 0	40.9
D 6-H 0	37.9	D 6-H 0	29.7	D 6-H 0	132	D 6-H 0	26.1	D 6-H 0	73.3	D 6-H 0	51.8
D 7-H 0	43.4	D 7-H 0	30.3	D 7-H 0	85.6	D 7-H 0	27.2	D 7-H 0	149	D 7-H 0	74.3
D 7-H 2	55.8	D 7-H 2	31.2	D 7-H 2	109	D 7-H 2	19.4	D 7-H 2	186	D 7-H 2	94.6
D 7-H 4	93.4	D 7-H 4	78.4	D 7-H 4	183	D 7-H 4	43.3	D 7-H 4	184	D 7-H 4	132
D 7-H 6	131	D 7-H 6	72.8	D 7-H 6	141	D 7-H 6	58.7	D 7-H 6	227	D 7-H 6	142
D 7-H 8	144	D 7-H 8	67.9	D 7-H 8	111	D 7-H 8	37.8	D 7-H 8	229	D 7-H 8	136
D 7-H 12	101	D 7-H 12	48.6	D 7-H 12	109	D 7-H 12	31.9	D 7-H 12	180	D 7-H 12	111
D 8-H 0	57.6	D 8-H 0	30.3	D 8-H 0	97.9	D 8-H 0	28.4	D 8-H 0	108	D 8-H 0	63.1
D 9-H 0	77.2	D 9-H 0	50.7	D 9-H 0	69.2	D 9-H 0	33.0	D 9-H 0	89.1	D 9-H 0	101
D 10-H 0	68.9	D 10-H 0	61.0	D 10-H 0	70.6	D 10-H 0	35.8	D 10-H 0	105	D 10-H 0	76.4
D 11-H 0	50.6	D 11-H 0	64.4	D 11-H 0	68.9	D 11-H 0	27.8	D 11-H 0	121	D 11-H 0	NS
D 12-H 0	51.3	D 12-H 0	69.7	D 12-H 0	73.1	D 12-H 0	34.8	D 12-H 0	112	D 12-H 0	NS
D 13-H 0	55.9	D 13-H 0	79.8	D 13-H 0	74.5	D 13-H 0	35.3	D 13-H 0	145	D 13-H 0	NS
D 14-H 0	58.6	D 14-H 0	52.8	D 14-H 0	124	D 14-H 0	51.6	D 14-H 0	142	D 14-H 0	NS
D 14-H 2	65.6	D 14-H 2	75.5	D 14-H 2	110	D 14-H 2	56.4	D 14-H 2	142	D 14-H 2	NS
D 14-H 4	*	D 14-H 4	83.9	D 14-H 4	205	D 14-H 4	116	D 14-H 4	265	D 14-H 4	NS
D 14-H 6	73.3	D 14-H 6	107	D 14-H 6	234	D 14-H 6	74.0	D 14-H 6	264	D 14-H 6	NS
D 14-H 8	68.2	D 14-H 8	123	D 14-H 8	166	D 14-H 8	67.4	D 14-H 8	213	D 14-H 8	NS
D 14-H 12	80.8	D 14-H 12	84.2	D 14-H 12	117	D 14-H 12	46.4	D 14-H 12	232	D 14-H 12	NS

001 (JKS)		002 (BSH)		04 (WPS)		005 (SGA)		007 (DAN)		008 (AYB)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 15-H 0	47.5	D 15-H 0	58.5	D 15-H 0	89.4	D 15-H 0	50.6	D 15-H 0	147	D 15-H 0	NS
D 16-H 0	33.1	D 16-H 0	46.1	D 16-H 0	76.8	D 16-H 0	50.0	D 16-H 0	191	D 16-H 0	NS
D 17-H 0	35.0	D 17-H 0	61.3	D 17-H 0	76.0	D 17-H 0	30.7	D 17-H 0	133	D 17-H 0	NS
D 18-H 0	59.7	D 18-H 0	99.6	D 18-H 0	70.6	D 18-H 0	50.2	D 18-H 0	234	D 18-H 0	NS
D 19-H 0	68.3	D 19-H 0	104	D 19-H 0	66.4	D 19-H 0	50.0	D 19-H 0	150	D 19-H 0	NS
D 20-H 0	80.6	D 20-H 0	125	D 20-H 0	81.4	D 20-H 0	39.4	D 20-H 0	160	D 20-H 0	NS
D 21-H 0	84.5	D 21-H 0	83.1	D 21-H 0	97.7	D 21-H 0	40.5	D 21-H 0	226	D 21-H 0	NS
D 21-H 2	92.8	D 21-H 2	139	D 21-H 2	137	D 21-H 2	75.1	D 21-H 2	272	D 21-H 2	NS
D 21-H 4	94.6	D 21-H 4	273	D 21-H 4	195	D 21-H 4	79.3	D 21-H 4	292	D 21-H 4	NS
D 21-H 6	127	D 21-H 6	369	D 21-H 6	224	D 21-H 6	95.3	D 21-H 6	312	D 21-H 6	NS
D 21-H 8	81.0	D 21-H 8	319	D 21-H 8	190	D 21-H 8	62.2	D 21-H 8	258	D 21-H 8	NS
D 21-H 12	96.8	D 21-H 12	263	D 21-H 12	146	D 21-H 12	54.3	D 21-H 12	250	D 21-H 12	NS
D 22-H 0	52.5	D 22-H 0	127	D 22-H 0	92.8	D 22-H 0	35.9	D 22-H 0	170	D 22-H 0	NS
D 25-H 0	70.5	D 25-H 0	108	D 25-H 0	84.0	D 25-H 0	31.3	D 25-H 0	162	D 25-H 0	NS
D 29-H 0	169	D 29-H 0	195	D 29-H 0	75.9	D 29-H 0	NS	D 29-H 0	123	D 29-H 0	NS
D 32-H 0	82.4	D 32-H 0	111	D 32-H 0	105	D 32-H 0	NS	D 32-H 0	155	D 32-H 0	NS
D 33-H 0	59.2	D 34-H 0	65.9	D 33-H 0	NS	D 33-H 0	NS	D 35-H 0	93.9	D 33-H 0	NS
D 36-H 0	54.6	D 35-H 0	60.8	D 36-H 0	59.1	D 36-H 0	NS	D 36-H 0	116	D 36-H 0	NS
D 39-H 0	54.4	D 39-H 0	NS	D 37-H 0	52.2	D 39-H 0	NS	D 39-H 0	206	D 39-H 0	NS
D 42-H 0	109	D 42-H 0	NS	D 42-H 0	NS	D 42-H 0	NS	D 42-H 0	182	D 42-H 0	NS
D 42-H 0.5	121	D 42-H 0.5	NS	D 42-H 0.5	NS	D 42-H 0.5	NS	D 42-H 0.5	123	D 42-H 0.5	NS
D 42-H 1	112	D 42-H 1	NS	D 42-H 1	NS	D 42-H 1	NS	D 42-H 1	150	D 42-H 1	NS
D 42-H 2	134	D 42-H 2	NS	D 42-H 2	NS	D 42-H 2	NS	D 42-H 2	151	D 42-H 2	NS
D 42-H 3	160	D 42-H 3	NS	D 42-H 3	NS	D 42-H 3	NS	D 42-H 3	165	D 42-H 3	NS
D 42-H 4	157	D 42-H 4	NS	D 42-H 4	NS	D 42-H 4	NS	D 42-H 4	204	D 42-H 4	NS
D 42-H 6	181	D 42-H 6	NS	D 42-H 6	NS	D 42-H 6	NS	D 42-H 6	172	D 42-H 6	NS
D 42-H 8	193	D 42-H 8	NS	D 42-H 8	NS	D 42-H 8	NS	D 42-H 8	202	D 42-H 8	NS
D 42-H 10	153	D 42-H 10	NS	D 42-H 10	NS	D 42-H 10	NS	D 42-H 10	224	D 42-H 10	NS
D 42-H 12	168	D 42-H 12	NS	D 42-H 12	NS	D 42-H 12	NS	D 42-H 12	201	D 42-H 12	NS
D 43-H 0	64.0	D 43-H 0	NS	D 43-H 0	NS	D 43-H 0	NS	D 43-H 0	175	D 43-H 0	NS
D 44-H 0	67.9	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS
D 45-H 0	46.3	D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	68.6	D 45-H 0	NS
D 48-H 0	46.7	D 48-H 0	NS	D 48-H 0	NS	D 48-H 0	NS	D 49-H 0	NS	D 48-H 0	NS
D 51-H 0	34.9	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	42.0	D 51-H 0	NS
D 54-H 0	29.9	D 54-H 0	NS	D 54-H 0	NS	D 54-H 0	14.2	D 54-H 0	46.6	D 54-H 0	NS
D 57-H 0	32.1	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	49.9	D 57-H 0	NS
D 72-H 0	33.8	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	29.2	D 72-H 0	NS
D 180-H 0	*	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	*	D 180-H 0	NS

009 (GRL)		010 (EYJ)		011 (CE)		014 (DLS)		015 (DMK)		016(LW)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	17.9	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*
D 1-H 2	12.6	D 1-H 2	19.6	D 1-H 2	34.8	D 1-H 2	11.2	D 1-H 2	23.9	D 1-H 2	18.1
D 1-H 3	70.9	D 1-H 3	63.2	D 1-H 3	31.2	D 1-H 3	24.8	D 1-H 3	28.6	D 1-H 3	38.7
D 1-H 4	152	D 1-H 4	103	D 1-H 4	80.0	D 1-H 4	44.0	D 1-H 4	44.0	D 1-H 4	40.3
D 1-H 6	122	D 1-H 6	86.9	D 1-H 6	42.2	D 1-H 6	49.2	D 1-H 6	43.7	D 1-H 6	21.7
D 1-H 8	103	D 1-H 8	75.5	D 1-H 8	41.0	D 1-H 8	50.4	D 1-H 8	38.6	D 1-H 8	19.8
D 1-H 10	65.0	D 1-H 10	58.1	D 1-H 10	39.9	D 1-H 10	50.7	D 1-H 10	31.9	D 1-H 10	20.1
D 1-H 12	53.8	D 1-H 12	43.5	D 1-H 12	28.4	D 1-H 12	34.5	D 1-H 12	22.3	D 1-H 12	20.6
D 2-H 0	21.0	D 2-H 0	23.3	D 2-H 0	17.1	D 2-H 0	18.9	D 2-H 0	13.0	D 2-H 0	11.0
D 3-H 0	20.6	D 3-H 0	20.0	D 3-H 0	83.1	D 3-H 0	16.9	D 3-H 0	39.1	D 3-H 0	18.9
D 4-H 0	24.2	D 4-H 0	28.4	D 4-H 0	44.8	D 4-H 0	28.4	D 4-H 0	33.7	D 4-H 0	28.5
D 4-H 2	42.8	D 4-H 2	32.9	D 4-H 2	63.4	D 4-H 2	39.8	D 4-H 2	61.1	D 4-H 2	51.2
D 4-H 4	109	D 4-H 4	70.9	D 4-H 4	134	D 4-H 4	48.7	D 4-H 4	70.6	D 4-H 4	94.1
D 4-H 6	95.8	D 4-H 6	63.2	D 4-H 6	103	D 4-H 6	68.0	D 4-H 6	70.2	D 4-H 6	68.6
D 4-H 8	60.5	D 4-H 8	49.2	D 4-H 8	99.4	D 4-H 8	44.8	D 4-H 8	59.7	D 4-H 8	55.7
D 4-H 12	38.6	D 4-H 12	34.1	D 4-H 12	79.3	D 4-H 12	38.6	D 4-H 12	47.3	D 4-H 12	46.9
D 5-H 0	27.0	D 5-H 0	24.3	D 5-H 0	48.5	D 5-H 0	28.7	D 5-H 0	33.2	D 5-H 0	35.2
D 6-H 0	36.7	D 6-H 0	20.5	D 6-H 0	57.6	D 6-H 0	47.1	D 6-H 0	35.1	D 6-H 0	42.7
D 7-H 0	40.9	D 7-H 0	19.8	D 7-H 0	32.5	D 7-H 0	52.5	D 7-H 0	34.6	D 7-H 0	53.1
D 7-H 2	36.1	D 7-H 2	43.5	D 7-H 2	55.4	D 7-H 2	40.9	D 7-H 2	58.5	D 7-H 2	83.3
D 7-H 4	122	D 7-H 4	63.3	D 7-H 4	109	D 7-H 4	66.0	D 7-H 4	100	D 7-H 4	153
D 7-H 6	122	D 7-H 6	65.4	D 7-H 6	85.8	D 7-H 6	73.3	D 7-H 6	98.2	D 7-H 6	189
D 7-H 8	94.1	D 7-H 8	51.7	D 7-H 8	75.0	D 7-H 8	53.3	D 7-H 8	72.6	D 7-H 8	128
D 7-H 12	64.8	D 7-H 12	31.4	D 7-H 12	59.3	D 7-H 12	45.6	D 7-H 12	68.8	D 7-H 12	87.3
D 8-H 0	44.6	D 8-H 0	29.1	D 8-H 0	33.3	D 8-H 0	39.2	D 8-H 0	34.8	D 8-H 0	49.1
D 9-H 0	57.4	D 9-H 0	81.7	D 9-H 0	43.5	D 9-H 0	50.3	D 9-H 0	30.4	D 9-H 0	57.5
D 10-H 0	51.6	D 10-H 0	62.0	D 10-H 0	34.1	D 10-H 0	96.8	D 10-H 0	47.1	D 10-H 0	55.4
D 11-H 0	60.3	D 11-H 0	45.1	D 11-H 0	41.7	D 11-H 0	99.7	D 11-H 0	50.4	D 11-H 0	58.5
D 12-H 0	65.4	D 12-H 0	58.1	D 12-H 0	41.3	D 12-H 0	99.5	D 12-H 0	49.7	D 12-H 0	57.1
D 13-H 0	58.9	D 13-H 0	47.6	D 13-H 0	58.5	D 13-H 0	71.6	D 13-H 0	35.1	D 13-H 0	67.5
D 14-H 0	53.1	D 14-H 0	56.2	D 14-H 0	43.9	D 14-H 0	73.3	D 14-H 0	53.1	D 14-H 0	64.8
D 14-H 2	74.7	D 14-H 2	167	D 14-H 2	67.2	D 14-H 2	99.8	D 14-H 2	94.8	D 14-H 2	73.8
D 14-H 4	166	D 14-H 4	334	D 14-H 4	216	D 14-H 4	168	D 14-H 4	107	D 14-H 4	107
D 14-H 6	162	D 14-H 6	259	D 14-H 6	302	D 14-H 6	176	D 14-H 6	138	D 14-H 6	132
D 14-H 8	166	D 14-H 8	283	D 14-H 8	324	D 14-H 8	182	D 14-H 8	121	D 14-H 8	90.9
D 14-H 12	132	D 14-H 12	178	D 14-H 12	187	D 14-H 12	104	D 14-H 12	91.5	D 14-H 12	101

009 (GRL)		010 (EYJ)		011 (CE)		014 (DLS)		015 (DMK)		016(LW)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 15-H 0	57.8	D 15-H 0	100	D 15-H 0	99.7	D 15-H 0	89.4	D 15-H 0	49.4	D 15-H 0	76.4
D 16-H 0	68.9	D 16-H 0	88.8	D 16-H 0	129	D 16-H 0	118	D 16-H 0	48.9	D 16-H 0	72.4
D 17-H 0	65.0	D 17-H 0	86.4	D 17-H 0	83.0	D 17-H 0	98.5	D 17-H 0	82.8	D 17-H 0	80.5
D 18-H 0	83.4	D 18-H 0	84.3	D 18-H 0	59.4	D 18-H 0	96.2	D 18-H 0	59.1	D 18-H 0	94.3
D 19-H 0	59.6	D 19-H 0	98.6	D 19-H 0	73.9	D 19-H 0	91.6	D 19-H 0	51.6	D 19-H 0	81.8
D 20-H 0	62.3	D 20-H 0	83.6	D 20-H 0	60.5	D 20-H 0	90.0	D 20-H 0	53.8	D 20-H 0	94.0
D 21-H 0	68.6	D 21-H 0	65.9	D 21-H 0	65.6	D 21-H 0	60.4	D 21-H 0	38.2	D 21-H 0	97.7
D 21-H 2	81.9	D 21-H 2	224	D 21-H 2	67.1	D 21-H 2	156	D 21-H 2	85.4	D 21-H 2	110
D 21-H 4	164	D 21-H 4	359	D 21-H 4	86.2	D 21-H 4	154	D 21-H 4	142	D 21-H 4	219
D 21-H 6	234	D 21-H 6	278	D 21-H 6	127	D 21-H 6	140	D 21-H 6	145	D 21-H 6	215
D 21-H 8	221	D 21-H 8	254	D 21-H 8	96.8	D 21-H 8	99.8	D 21-H 8	125	D 21-H 8	152
D 21-H 12	111	D 21-H 12	173	D 21-H 12	81.4	D 21-H 12	86.2	D 21-H 12	89.6	D 21-H 12	151
D 22-H 0	91.2	D 22-H 0	95.1	D 22-H 0	56.1	D 22-H 0	74.7	D 22-H 0	49.7	D 22-H 0	91.5
D 25-H 0	42.5	D 25-H 0	98.4	D 25-H 0	54.1	D 25-H 0	126	D 25-H 0	40.1	D 25-H 0	85.9
D 29-H 0	58.1	D 29-H 0	87.9	D 29-H 0	46.4	D 29-H 0	272	D 29-H 0	86.2	D 29-H 0	69.8
D 32-H 0	86.4	D 32-H 0	136	D 32-H 0	63.4	D 32-H 0	215	D 32-H 0	94.5	D 32-H 0	138
D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS
D 36-H 0	42.0	D 36-H 0	118	D 36-H 0	NS	D 36-H 0	206	D 36-H 0	77.9	D 36-H 0	152
D 39-H 0	58.4	D 39-H 0	94.6	D 39-H 0	72.3	D 39-H 0	223	D 39-H 0	75.4	D 39-H 0	242
D 42-H 0	56.5	D 42-H 0	67.8	D 42-H 0	54.5	D 42-H 0	NS	D 42-H 0	76.1	D 42-H 0	87.7
D 42-H 0.5	54.9	D 42-H 0.5	83.7	D 42-H 0.5	49.4	D 42-H 0.5	NS	D 42-H 0.5	90.5	D 42-H 0.5	108
D 42-H 1	46.0	D 42-H 1	54.5	D 42-H 1	60.3	D 42-H 1	NS	D 42-H 1	100	D 42-H 1	90.5
D 42-H 2	104	D 42-H 2	165	D 42-H 2	142	D 42-H 2	NS	D 42-H 2	108	D 42-H 2	120
D 42-H 3	81.0	D 42-H 3	435	D 42-H 3	176	D 42-H 3	NS	D 42-H 3	151	D 42-H 3	208
D 42-H 4	88.0	D 42-H 4	318	D 42-H 4	205	D 42-H 4	NS	D 42-H 4	138	D 42-H 4	242
D 42-H 6	120	D 42-H 6	303	D 42-H 6	135	D 42-H 6	NS	D 42-H 6	123	D 42-H 6	183
D 42-H 8	90.9	D 42-H 8	204	D 42-H 8	111	D 42-H 8	NS	D 42-H 8	124	D 42-H 8	143
D 42-H 10	72.9	D 42-H 10	259	D 42-H 10	155	D 42-H 10	NS	D 42-H 10	114	D 42-H 10	178
D 42-H 12	70.8	D 42-H 12	203	D 42-H 12	92.9	D 42-H 12	NS	D 42-H 12	128	D 42-H 12	119
D 43-H 0	54.9	D 43-H 0	127	D 43-H 0	54.2	D 43-H 0	NS	D 43-H 0	89.1	D 43-H 0	114
D 44-H 0	37.3	D 44-H 0	58.9	D 44-H 0	43.6	D 44-H 0	NS	D 44-H 0	65.8	D 44-H 0	114
D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	38.5	D 45-H 0	NS	D 45-H 0	46.2	D 45-H 0	NS
D 48-H 0	25.8	D 49-H 0	43.2	D 48-H 0	NS	D 48-H 0	NS	D 48-H 0	33.0	D 48-H 0	NS
D 51-H 0	17.8	D 51-H 0	36.1	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	26.2	D 51-H 0	NS
D 54-H 0	22.0	D 54-H 0	24.7	D 54-H 0	NS	D 54-H 0	NS	D 54-H 0	31.5	D 54-H 0	NS
D 57-H 0	20.1	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	20.2	D 57-H 0	NS
D 72-H 0	13.2	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	22.1	D 76-H 0	21.1
D 180-H 0	*	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	*	D 180-H 0	*

Final Data Hal/P 95-4 Chiral

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17		18		19		20	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	26.6	D 1-H 1	*
D 1-H 2	*	D 1-H 2	24.1	D 1-H 2	40.7	D 1-H 2	*
D 1-H 3	*	D 1-H 3	82.5	D 1-H 3	48.0	D 1-H 3	14.8
D 1-H 4	*	D 1-H 4	147	D 1-H 4	40.9	D 1-H 4	24.2
D 1-H 6	*	D 1-H 6	105	D 1-H 6	60.4	D 1-H 6	19.5
		D 1-H 8	85.9	D 1-H 8	37.8	D 1-H 8	12.7
		D 1-H 10	76.2	D 1-H 10	45.2	D 1-H 10	*
		D 1-H 12	61.2	D 1-H 12	34.3	D 1-H 12	*
		D 2-H 0	60.6	D 2-H 0	26.6	D 2-H 0	*
		D 3-H 0	29.3	D 3-H 0	44.7	D 3-H 0	*
		D 4-H 0	67.1	D 4-H 0	50.4	D 4-H 0	15.0
		D 4-H 2	74.3	D 4-H 2	65.0	D 4-H 2	22.3
		D 4-H 4	116	D 4-H 4	151	D 4-H 4	*
		D 4-H 6	156	D 4-H 6	145	D 4-H 6	*
		D 4-H 8	114	D 4-H 8	110	D 4-H 8	36.0
		D 4-H 12	95.9	D 4-H 12	66.0	D 4-H 12	30.2
		D 5-H 0	60.5	D 5-H 0	50.5	D 5-H 0	17.6
		D 6-H 0	66.0	D 6-H 0	52.5	D 6-H 0	19.2
		D 7-H 0	114	D 7-H 0	53.6	D 7-H 0	20.1
		D 7-H 2	82.4	D 7-H 2	104	D 7-H 2	30.0
		D 7-H 4	224	D 7-H 4	155	D 7-H 4	82.2
		D 7-H 6	226	D 7-H 6	130	D 7-H 6	39.5
		D 7-H 8	87.4	D 7-H 8	87.8	D 7-H 8	38.2
		D 7-H 12	141	D 7-H 12	81.9	D 7-H 12	30.0
		D 8-H 0	87.9	D 8-H 0	61.0	D 8-H 0	24.2
		D 9-H 0	82.2	D 9-H 0	44.5	D 9-H 0	23.2
		D 10-H 0	91.2	D 10-H 0	64.0	D 10-H 0	28.2
		D 11-H 0	85.4	D 11-H 0	57.5	D 11-H 0	23.1
		D 12-H 0	103	D 12-H 0	60.9	D 12-H 0	20.4
		D 13-H 0	85.0	D 13-H 0	57.8	D 13-H 0	29.3
		D 14-H 0	87.9	D 14-H 0	139	D 14-H 0	29.7
		D 14-H 2	171	D 15-H 0	59.9	D 14-H 2	28.2
		D 14-H 4	166	D 15-H 2	98.1	D 14-H 4	47.1
		D 14-H 6	149	D 15-H 4	210	D 14-H 6	40.5
		D 14-H 8	118	D 15-H 6	197	D 14-H 8	55.2
		D 14-H 12	125	D 15-H 8	164	D 14-H 12	50.0

17		18		19		20	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
		D 15-H 0	95.7	D 15-H 12	121	D 15-H 0	25.8
		D 16-H 0	104	D 16-H 0	67.6	D 16-H 0	25.4
		D 17-H 0	106	D 17-H 0	72.0	D 17-H 0	25.1
		D 18-H 0	90.4	D 18-H 0	78.1	D 18-H 0	14.4
		D 19-H 0	86.5	D 19-H 0	86.7	D 19-H 0	*
		D 20-H 0	81.3	D 20-H 0	110	D 20-H 0	32.9
		D 21-H 0	90.8	D 21-H 0	89.9	D 21-H 0	36.5
		D 21-H 2	113	D 21-H 2	51.1	D 21-H 2	41.6
		D 21-H 4	147	D 21-H 4	123	D 21-H 4	83.8
		D 21-H 6	186	D 21-H 6	76.3	D 21-H 6	51.7
		D 21-H 8	166	D 21-H 8	77.9	D 21-H 8	56.0
		D 21-H 12	116	D 21-H 12	43.4	D 21-H 12	45.6
		D 22-H 0	93.0	D 22-H 0	33.0	D 22-H 0	19.2
		D 25-H 0	56.7	D 25-H 0	29.6	D 25-H 0	40.8
		D 29-H 0	70.1	D 29-H 0	37.3	D 29-H 0	78.4
		D 32-H 0	157	D 32-H 0	40.1	D 30-H 0	67.7
		D 33-H 0	NS	D 33-H 0	52.0	D 32-H 0	41.1
		D 36-H 0	NS	D 36-H 0	49.6	D 33-H 0	35.9
		D 39-H 0	NS	D 39-H 0	55.2	D 35-H 0	36.9
		D 42-H 0	213	D 42-H 0	44.9	D 39-H 0	22.4
		D 42-H 0.5	215	D 42-H 0.5	45.9	D 42-H 0	33.8
		D 42-H 1	201	D 42-H 1	56.4	D 42-H 0.5	27.0
		D 42-H 2	209	D 42-H 2	74.2	D 42-H 1	40.8
		D 42-H 3	219	D 42-H 3	105	D 42-H 2	35.7
		D 42-H 4	293	D 42-H 4	106	D 42-H 3	71.0
		D 42-H 6	311	D 42-H 6	149	D 42-H 4	66.1
		D 42-H 8	260	D 42-H 8	101	D 42-H 6	80.3
		D 42-H 10	237	D 42-H 10	82.2	D 42-H 8	63.6
		D 42-H 12	247	D 42-H 12	80.6	D 42-H 10	57.3
		D 43-H 0	165	D 43-H 0	61.5	D 42-H 12	49.5
		D 44-H 0	NS	D 44-H 0	40.8	D 43-H 0	34.3
		D 45-H 0	110	D 45-H 0	32.0	D 44-H 0	21.7
		D 48-H 0	54.8	D 48-H 0	27.8	D 45-H 0	17.4
		D 52-H 0	58.9	D 51-H 0	19.5	D 48-H 0	*
		D 54-H 0	42.3	D 54-H 0	15.8	D 51-H 0	*
		D 57-H 0	42.7	D 57-H 0	13.1	D 60-H 0	*
		D 72-H 0	27.7	D 72-H 0	*	D 72-H 0	18.9
		D 180-H 0	NS	D 180-H 0	*	D 180-H 0	12.9

Final Data Hal/P 95-4 Chiral

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001 (JKS)		002 (BSH)		04 (WPS)		005 (SGA)		007 (DAN)		008 (AYB)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	10.7	D 1-H 1	*	D 1-H 1	13.9
D 1-H 2	*	D 1-H 2	*	D 1-H 2	12.6	D 1-H 2	17.2	D 1-H 2	*	D 1-H 2	32.9
D 1-H 3	*	D 1-H 3	*	D 1-H 3	37.9	D 1-H 3	15.1	D 1-H 3	10.6	D 1-H 3	50.3
D 1-H 4	*	D 1-H 4	*	D 1-H 4	20.5	D 1-H 4	*	D 1-H 4	18.7	D 1-H 4	57.7
D 1-H 6	*	D 1-H 6	22.7	D 1-H 6	32.8	D 1-H 6	18.6	D 1-H 6	15.6	D 1-H 6	113
D 1-H 8	*	D 1-H 8	35.1	D 1-H 8	19.0	D 1-H 8	10.3	D 1-H 8	15.6	D 1-H 8	69.7
D 1-H 10	10.4	D 1-H 10	20.1	D 1-H 10	*	D 1-H 10	16.3	D 1-H 10	14.1	D 1-H 10	41.7
D 1-H 12	*	D 1-H 12	12.3	D 1-H 12	10.2	D 1-H 12	*	D 1-H 12	12.5	D 1-H 12	40.3
D 2-H 0	*	D 2-H 0	*	D 2-H 0	*	D 2-H 0	*	D 2-H 0	10.6	D 2-H 0	13.6
D 3-H 0	*	D 3-H 0	*	D 3-H 0	10.7	D 3-H 0	*	D 3-H 0	36.6	D 3-H 0	21.7
D 4-H 0	*	D 4-H 0	16.5	D 4-H 0	41.8	D 4-H 0	13.1	D 4-H 0	48.3	D 4-H 0	19.9
D 4-H 2	22.8	D 4-H 2	37.1	D 4-H 2	356	D 4-H 2	15.1	D 4-H 2	326	D 4-H 2	49.8
D 4-H 4	41.7	D 4-H 4	55.5	D 4-H 4	329	D 4-H 4	44.2	D 4-H 4	570	D 4-H 4	113
D 4-H 6	37.7	D 4-H 6	38.7	D 4-H 6	310	D 4-H 6	27.0	D 4-H 6	343	D 4-H 6	75.4
D 4-H 8	17.1	D 4-H 8	26.3	D 4-H 8	191	D 4-H 8	32.6	D 4-H 8	223	D 4-H 8	66.6
D 4-H 12	30.7	D 4-H 12	18.1	D 4-H 12	146	D 4-H 10	25.5	D 4-H 12	158	D 4-H 12	76.7
D 5-H 0	22.5	D 5-H 0	16.6	D 5-H 0	57.9	D 5-H 0	11.7	D 5-H 0	56.5	D 5-H 0	22.4
D 6-H 0	16.5	D 6-H 0	16.6	D 6-H 0	74.4	D 6-H 0	*	D 6-H 0	45.0	D 6-H 0	25.1
D 7-H 0	18.2	D 7-H 0	14.8	D 7-H 0	38.6	D 7-H 0	11.2	D 7-H 0	85.6	D 7-H 0	41.7
D 7-H 2	33.7	D 7-H 2	19.2	D 7-H 2	60.3	D 7-H 2	11.4	D 7-H 2	128	D 7-H 2	63.0
D 7-H 4	59.3	D 7-H 4	53.7	D 7-H 4	107	D 7-H 4	23.9	D 7-H 4	127	D 7-H 4	85.4
D 7-H 6	73.1	D 7-H 6	43.0	D 7-H 6	72.5	D 7-H 6	31.8	D 7-H 6	152	D 7-H 6	83.8
D 7-H 8	79.6	D 7-H 8	41.4	D 7-H 8	55.7	D 7-H 8	18.6	D 7-H 8	152	D 7-H 8	79.3
D 7-H 12	50.6	D 7-H 12	25.9	D 7-H 12	49.7	D 7-H 12	14.8	D 7-H 12	117	D 7-H 12	62.1
D 8-H 0	27.4	D 8-H 0	15.7	D 8-H 0	35.0	D 8-H 0	12.3	D 8-H 0	67.3	D 8-H 0	31.7
D 9-H 0	33.1	D 9-H 0	27.0	D 9-H 0	30.0	D 9-H 0	15.4	D 9-H 0	56.0	D 9-H 0	53.4
D 10-H 0	28.0	D 10-H 0	27.8	D 10-H 0	29.8	D 10-H 0	14.0	D 10-H 0	58.8	D 10-H 0	40.8
D 11-H 0	20.4	D 11-H 0	32.7	D 11-H 0	30.3	D 11-H 0	11.4	D 11-H 0	71.5	D 11-H 0	NS
D 12-H 0	20.8	D 12-H 0	33.2	D 12-H 0	32.2	D 12-H 0	14.9	D 12-H 0	63.8	D 12-H 0	NS
D 13-H 0	25.4	D 13-H 0	43.4	D 13-H 0	28.1	D 13-H 0	16.0	D 13-H 0	74.5	D 13-H 0	NS
D 14-H 0	25.5	D 14-H 0	25.0	D 14-H 0	52.7	D 14-H 0	26.9	D 14-H 0	75.9	D 14-H 0	NS
D 14-H 2	30.3	D 14-H 2	46.7	D 14-H 2	50.2	D 14-H 2	35.1	D 14-H 2	84.8	D 14-H 2	NS
D 14-H 4	*	D 14-H 4	51.8	D 14-H 4	104	D 14-H 4	66.5	D 14-H 4	161	D 14-H 4	NS
D 14-H 6	37.4	D 14-H 6	62.5	D 14-H 6	119	D 14-H 6	41.3	D 14-H 6	154	D 14-H 6	NS
D 14-H 8	36.2	D 14-H 8	71.9	D 14-H 8	79.4	D 14-H 8	35.2	D 14-H 8	115	D 14-H 8	NS
D 14-H 12	35.0	D 14-H 12	46.0	D 14-H 12	51.6	D 14-H 12	23.0	D 14-H 12	124	D 14-H 12	NS

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Final Data Hal/P 95-4 Chiral

001 (JKS)		002 (BSH)		04 (WPS)		005 (SGA)		007 (DAN)		008 (AYB)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 15-H 0	20.8	D 15-H 0	27.9	D 15-H 0	35.2	D 15-H 0	24.8	D 15-H 0	72.2	D 15-H 0	NS
D 16-H 0	14.3	D 16-H 0	21.3	D 16-H 0	33.0	D 16-H 0	20.8	D 16-H 0	99.4	D 16-H 0	NS
D 17-H 0	14.3	D 17-H 0	29.7	D 17-H 0	33.9	D 17-H 0	14.6	D 17-H 0	62.5	D 17-H 0	NS
D 18-H 0	29.6	D 18-H 0	54.2	D 18-H 0	28.4	D 18-H 0	24.1	D 18-H 0	119	D 18-H 0	NS
D 19-H 0	32.8	D 19-H 0	59.8	D 19-H 0	28.1	D 19-H 0	23.1	D 19-H 0	72.4	D 19-H 0	NS
D 20-H 0	36.6	D 20-H 0	66.4	D 20-H 0	31.5	D 20-H 0	18.2	D 20-H 0	79.0	D 20-H 0	NS
D 21-H 0	37.4	D 21-H 0	40.8	D 21-H 0	38.6	D 21-H 0	19.0	D 21-H 0	110	D 21-H 0	NS
D 21-H 2	41.0	D 21-H 2	99.5	D 21-H 2	62.7	D 21-H 2	46.2	D 21-H 2	156	D 21-H 2	NS
D 21-H 4	51.7	D 21-H 4	240	D 21-H 4	113	D 21-H 4	41.9	D 21-H 4	152	D 21-H 4	NS
D 21-H 6	70.0	D 21-H 6	298	D 21-H 6	122	D 21-H 6	55.1	D 21-H 6	171	D 21-H 6	NS
D 21-H 8	40.6	D 21-H 8	239	D 21-H 8	95.4	D 21-H 8	32.9	D 21-H 8	130	D 21-H 8	NS
D 21-H 12	51.7	D 21-H 12	179	D 21-H 12	63.9	D 21-H 12	28.7	D 21-H 12	121	D 21-H 12	NS
D 22-H 0	24.4	D 22-H 0	75.4	D 22-H 0	36.9	D 22-H 0	16.8	D 22-H 0	80.8	D 22-H 0	NS
D 25-H 0	31.1	D 25-H 0	60.4	D 25-H 0	32.8	D 25-H 0	14.8	D 25-H 0	73.2	D 25-H 0	NS
D 29-H 0	93.7	D 29-H 0	122	D 29-H 0	28.5	D 29-H 0	NS	D 29-H 0	57.0	D 29-H 0	NS
D 32-H 0	35.8	D 32-H 0	60.6	D 32-H 0	40.2	D 32-H 0	NS	D 32-H 0	78.0	D 32-H 0	NS
D 33-H 0	25.9	D 34-H 0	31.2	D 33-H 0	NS	D 33-H 0	NS	D 35-H 0	55.7	D 33-H 0	NS
D 36-H 0	26.7	D 35-H 0	33.2	D 36-H 0	23.5	D 36-H 0	NS	D 36-H 0	60.2	D 36-H 0	NS
D 39-H 0	23.7	D 39-H 0	NS	D 37-H 0	19.2	D 39-H 0	NS	D 39-H 0	97.6	D 39-H 0	NS
D 42-H 0	50.2	D 42-H 0	NS	D 42-H 0	NS	D 42-H 0	NS	D 42-H 0	80.6	D 42-H 0	NS
D 42-H 0.5	55.8	D 42-H 0.5	NS	D 42-H 0.5	NS	D 42-H 0.5	NS	D 42-H 0.5	56.2	D 42-H 0.5	NS
D 42-H 1	56.0	D 42-H 1	NS	D 42-H 1	NS	D 42-H 1	NS	D 42-H 1	71.5	D 42-H 1	NS
D 42-H 2	68.0	D 42-H 2	NS	D 42-H 2	NS	D 42-H 2	NS	D 42-H 2	79.1	D 42-H 2	NS
D 42-H 3	88.2	D 42-H 3	NS	D 42-H 3	NS	D 42-H 3	NS	D 42-H 3	81.7	D 42-H 3	NS
D 42-H 4	91.4	D 42-H 4	NS	D 42-H 4	NS	D 42-H 4	NS	D 42-H 4	106	D 42-H 4	NS
D 42-H 6	106	D 42-H 6	NS	D 42-H 6	NS	D 42-H 6	NS	D 42-H 6	83.2	D 42-H 6	NS
D 42-H 8	110	D 42-H 8	NS	D 42-H 8	NS	D 42-H 8	NS	D 42-H 8	95.6	D 42-H 8	NS
D 42-H 10	93.7	D 42-H 10	NS	D 42-H 10	NS	D 42-H 10	NS	D 42-H 10	92.9	D 42-H 10	NS
D 42-H 12	90.5	D 42-H 12	NS	D 42-H 12	NS	D 42-H 12	NS	D 42-H 12	88.5	D 42-H 12	NS
D 43-H 0	35.2	D 43-H 0	NS	D 43-H 0	NS	D 43-H 0	NS	D 43-H 0	72.7	D 43-H 0	NS
D 44-H 0	27.6	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS
D 45-H 0	18.8	D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	45.2	D 45-H 0	NS
D 48-H 0	18.4	D 48-H 0	NS	D 48-H 0	NS	D 48-H 0	NS	D 49-H 0	NS	D 48-H 0	NS
D 51-H 0	12.7	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	18.7	D 51-H 0	NS
D 54-H 0	10.4	D 54-H 0	NS	D 54-H 0	NS	D 54-H 0	*	D 54-H 0	21.1	D 54-H 0	NS
D 57-H 0	12.3	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	21.6	D 57-H 0	NS
D 72-H 0	15.2	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	23.4	D 72-H 0	NS
D 180-H 0	*	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	*	D 180-H 0	NS

Final Data Hal/P 95-4 Chiral

5/4/98

009(GRL)		010 (EYJ)		011 (CE)		014 (DLS)		015 (DMK)		016(LW)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	16.9	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*
D 1-H 2	11.2	D 1-H 2	28.8	D 1-H 2	27.4	D 1-H 2	*	D 1-H 2	19.6	D 1-H 2	12.9
D 1-H 3	61.8	D 1-H 3	72.8	D 1-H 3	23.0	D 1-H 3	13.7	D 1-H 3	19.9	D 1-H 3	25.9
D 1-H 4	132	D 1-H 4	88.5	D 1-H 4	50.9	D 1-H 4	19.5	D 1-H 4	26.9	D 1-H 4	24.4
D 1-H 6	102	D 1-H 6	66.0	D 1-H 6	24.8	D 1-H 6	22.3	D 1-H 6	23.9	D 1-H 6	11.1
D 1-H 8	81.4	D 1-H 8	61.7	D 1-H 8	22.9	D 1-H 8	20.7	D 1-H 8	20.7	D 1-H 8	*
D 1-H 10	47.0	D 1-H 10	56.2	D 1-H 10	23.0	D 1-H 10	19.7	D 1-H 10	14.1	D 1-H 10	10.2
D 1-H 12	37.4	D 1-H 12	47.6	D 1-H 12	16.5	D 1-H 12	12.7	D 1-H 12	*	D 1-H 12	*
D 2-H 0	15.2	D 2-H 0	21.4	D 2-H 0	*	D 2-H 0	*	D 2-H 0	*	D 2-H 0	*
D 3-H 0	13.4	D 3-H 0	12.4	D 3-H 0	47.5	D 3-H 0	*	D 3-H 0	18.8	D 3-H 0	*
D 4-H 0	16.5	D 4-H 0	23.3	D 4-H 0	22.1	D 4-H 0	*	D 4-H 0	14.0	D 4-H 0	13.6
D 4-H 2	29.5	D 4-H 2	30.0	D 4-H 2	44.6	D 4-H 2	19.2	D 4-H 2	37.1	D 4-H 2	30.4
D 4-H 4	73.3	D 4-H 4	56.9	D 4-H 4	96.9	D 4-H 4	23.7	D 4-H 4	39.7	D 4-H 4	54.3
D 4-H 6	63.6	D 4-H 6	46.7	D 4-H 6	69.9	D 4-H 6	29.1	D 4-H 6	36.5	D 4-H 6	37.2
D 4-H 8	42.5	D 4-H 8	37.1	D 4-H 8	65.6	D 4-H 8	15.0	D 4-H 8	28.3	D 4-H 8	28.6
D 4-H 12	24.1	D 4-H 12	23.0	D 4-H 12	48.7	D 4-H 12	13.2	D 4-H 12	21.0	D 4-H 12	23.4
D 5-H 0	17.5	D 5-H 0	15.4	D 5-H 0	29.5	D 5-H 0	10.3	D 5-H 0	12.2	D 5-H 0	16.6
D 6-H 0	20.5	D 6-H 0	16.5	D 6-H 0	34.4	D 6-H 0	17.3	D 6-H 0	13.0	D 6-H 0	20.7
D 7-H 0	23.4	D 7-H 0	14.2	D 7-H 0	21.6	D 7-H 0	17.6	D 7-H 0	12.2	D 7-H 0	24.1
D 7-H 2	21.4	D 7-H 2	37.5	D 7-H 2	42.4	D 7-H 2	23.9	D 7-H 2	33.8	D 7-H 2	50.3
D 7-H 4	86.7	D 7-H 4	54.6	D 7-H 4	79.1	D 7-H 4	27.0	D 7-H 4	57.7	D 7-H 4	87.3
D 7-H 6	87.0	D 7-H 6	63.9	D 7-H 6	56.7	D 7-H 6	29.6	D 7-H 6	54.4	D 7-H 6	105
D 7-H 8	60.8	D 7-H 8	43.9	D 7-H 8	49.1	D 7-H 8	19.9	D 7-H 8	36.9	D 7-H 8	66.8
D 7-H 12	43.5	D 7-H 12	25.1	D 7-H 12	37.5	D 7-H 12	13.6	D 7-H 12	27.5	D 7-H 12	43.7
D 8-H 0	28.0	D 8-H 0	21.4	D 8-H 0	20.0	D 8-H 0	12.9	D 8-H 0	14.4	D 8-H 0	23.4
D 9-H 0	35.1	D 9-H 0	50.4	D 9-H 0	25.8	D 9-H 0	16.9	D 9-H 0	13.3	D 9-H 0	26.1
D 10-H 0	29.0	D 10-H 0	45.4	D 10-H 0	21.1	D 10-H 0	33.6	D 10-H 0	22.1	D 10-H 0	24.9
D 11-H 0	36.9	D 11-H 0	32.1	D 11-H 0	26.2	D 11-H 0	33.3	D 11-H 0	23.7	D 11-H 0	27.9
D 12-H 0	43.4	D 12-H 0	34.7	D 12-H 0	23.7	D 12-H 0	30.0	D 12-H 0	22.8	D 12-H 0	28.7
D 13-H 0	33.1	D 13-H 0	28.0	D 13-H 0	36.4	D 13-H 0	23.9	D 13-H 0	17.7	D 13-H 0	29.4
D 14-H 0	30.0	D 14-H 0	45.2	D 14-H 0	31.3	D 14-H 0	22.8	D 14-H 0	24.8	D 14-H 0	27.9
D 14-H 2	49.0	D 14-H 2	140	D 14-H 2	52.7	D 14-H 2	40.4	D 14-H 2	61.6	D 14-H 2	38.7
D 14-H 4	124	D 14-H 4	359	D 14-H 4	205	D 14-H 4	82.6	D 14-H 4	65.2	D 14-H 4	57.6
D 14-H 6	119	D 14-H 6	192	D 14-H 6	264	D 14-H 6	87.9	D 14-H 6	75.5	D 14-H 6	68.9
D 14-H 8	130	D 14-H 8	196	D 14-H 8	269	D 14-H 8	84.0	D 14-H 8	64.9	D 14-H 8	46.8
D 14-H 12	92.1	D 14-H 12	109	D 14-H 12	159	D 14-H 12	42.0	D 14-H 12	41.1	D 14-H 12	44.2

009(GRL)		010 (EYJ)		011 (CE)		014 (DLS)		015 (DMK)		016(LW)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 15-H 0	36.8	D 15-H 0	57.3	D 15-H 0	61.6	D 15-H 0	34.7	D 15-H 0	21.1	D 15-H 0	31.1
D 16-H 0	43.7	D 16-H 0	60.3	D 16-H 0	85.1	D 16-H 0	45.7	D 16-H 0	22.9	D 16-H 0	29.2
D 17-H 0	39.7	D 17-H 0	50.4	D 17-H 0	46.6	D 17-H 0	31.7	D 17-H 0	38.9	D 17-H 0	33.6
D 18-H 0	52.1	D 18-H 0	47.2	D 18-H 0	32.1	D 18-H 0	28.3	D 18-H 0	42.7	D 18-H 0	37.6
D 19-H 0	35.5	D 19-H 0	57.7	D 19-H 0	41.7	D 19-H 0	29.1	D 19-H 0	23.1	D 19-H 0	35.1
D 20-H 0	37.8	D 20-H 0	45.4	D 20-H 0	30.9	D 20-H 0	27.6	D 20-H 0	29.5	D 20-H 0	40.9
D 21-H 0	41.2	D 21-H 0	37.4	D 21-H 0	34.6	D 21-H 0	18.4	D 21-H 0	17.0	D 21-H 0	38.6
D 21-H 2	54.1	D 21-H 2	200	D 21-H 2	40.4	D 21-H 2	69.5	D 21-H 2	49.4	D 21-H 2	58.3
D 21-H 4	120	D 21-H 4	333	D 21-H 4	58.9	D 21-H 4	65.1	D 21-H 4	94.3	D 21-H 4	125
D 21-H 6	166	D 21-H 6	192	D 21-H 6	87.4	D 21-H 6	62.2	D 21-H 6	92.5	D 21-H 6	120
D 21-H 8	155	D 21-H 8	172	D 21-H 8	65.6	D 21-H 8	40.9	D 21-H 8	76.3	D 21-H 8	80.8
D 21-H 12	73.3	D 21-H 12	109	D 21-H 12	50.8	D 21-H 12	29.1	D 21-H 12	50.1	D 21-H 12	69.6
D 22-H 0	55.6	D 22-H 0	51.1	D 22-H 0	29.9	D 22-H 0	22.6	D 22-H 0	24.8	D 22-H 0	39.2
D 25-H 0	25.4	D 25-H 0	62.7	D 25-H 0	29.1	D 25-H 0	38.3	D 25-H 0	18.5	D 25-H 0	36.1
D 29-H 0	36.8	D 29-H 0	53.5	D 29-H 0	25.8	D 29-H 0	91.3	D 29-H 0	43.9	D 29-H 0	30.7
D 32-H 0	60.2	D 32-H 0	82.1	D 32-H 0	37.8	D 32-H 0	72.6	D 32-H 0	48.7	D 32-H 0	66.9
D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS
D 36-H 0	20.6	D 36-H 0	64.5	D 36-H 0	NS	D 36-H 0	61.6	D 36-H 0	34.5	D 36-H 0	68.4
D 39-H 0	32.8	D 39-H 0	47.4	D 39-H 0	42.0	D 39-H 0	70.6	D 39-H 0	36.5	D 39-H 0	115
D 42-H 0	34.3	D 42-H 0	35.6	D 42-H 0	30.1	D 42-H 0	NS	D 42-H 0	35.8	D 42-H 0	39.7
D 42-H 0.5	29.4	D 42-H 0.5	44.0	D 42-H 0.5	27.3	D 42-H 0.5	NS	D 42-H 0.5	44.8	D 42-H 0.5	47.8
D 42-H 1	29.9	D 42-H 1	37.8	D 42-H 1	39.6	D 42-H 1	NS	D 42-H 1	54.1	D 42-H 1	41.2
D 42-H 2	68.9	D 42-H 2	124	D 42-H 2	115	D 42-H 2	NS	D 42-H 2	56.8	D 42-H 2	63.3
D 42-H 3	50.9	D 42-H 3	468	D 42-H 3	127	D 42-H 3	NS	D 42-H 3	86.0	D 42-H 3	114
D 42-H 4	52.6	D 42-H 4	261	D 42-H 4	147	D 42-H 4	NS	D 42-H 4	73.5	D 42-H 4	137
D 42-H 6	73.2	D 42-H 6	234	D 42-H 6	93.6	D 42-H 6	NS	D 42-H 6	64.4	D 42-H 6	96.5
D 42-H 8	49.6	D 42-H 8	150	D 42-H 8	67.8	D 42-H 8	NS	D 42-H 8	66.2	D 42-H 8	71.4
D 42-H 10	43.2	D 42-H 10	184	D 42-H 10	98.2	D 42-H 10	NS	D 42-H 10	58.1	D 42-H 10	86.3
D 42-H 12	40.4	D 42-H 12	128	D 42-H 12	55.2	D 42-H 12	NS	D 42-H 12	66.9	D 42-H 12	57.8
D 43-H 0	29.4	D 43-H 0	80.5	D 43-H 0	31.5	D 43-H 0	NS	D 43-H 0	42.7	D 43-H 0	49.6
D 44-H 0	21.1	D 44-H 0	33.3	D 44-H 0	24.3	D 44-H 0	NS	D 44-H 0	32.0	D 44-H 0	46.6
D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	22.3	D 45-H 0	NS	D 45-H 0	22.1	D 45-H 0	NS
D 48-H 0	15.0	D 49-H 0	37.0	D 48-H 0	NS	D 48-H 0	NS	D 48-H 0	12.8	D 48-H 0	NS
D 51-H 0	*	D 51-H 0	30.9	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	*	D 51-H 0	NS
D 54-H 0	12.2	D 54-H 0	23.4	D 54-H 0	NS	D 54-H 0	NS	D 54-H 0	12.4	D 54-H 0	NS
D 57-H 0	10.7	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	*	D 57-H 0	NS
D 72-H 0	56.5	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	*	D 76-H 0	*
D 180-H 0	44.8	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	*	D 180-H 0	*

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Final Data Hal/P 95-4 Chiral

17		18		19		20	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	23.2	D 1-H 1	*
D 1-H 2	*	D 1-H 2	20.5	D 1-H 2	34.7	D 1-H 2	*
D 1-H 3	*	D 1-H 3	75.1	D 1-H 3	34.5	D 1-H 3	13.8
D 1-H 4	*	D 1-H 4	128	D 1-H 4	27.8	D 1-H 4	18.1
D 1-H 6	*	D 1-H 6	83.2	D 1-H 6	37.4	D 1-H 6	12.9
		D 1-H 8	58.9	D 1-H 8	20.8	D 1-H 8	*
		D 1-H 10	49.3	D 1-H 10	24.7	D 1-H 10	*
		D 1-H 12	38.3	D 1-H 12	19.2	D 1-H 12	*
		D 2-H 0	26.6	D 2-H 0	13.4	D 2-H 0	*
		D 3-H 0	15.0	D 3-H 0	26.9	D 3-H 0	*
		D 4-H 0	31.1	D 4-H 0	29.0	D 4-H 0	11.5
		D 4-H 2	42.3	D 4-H 2	43.1	D 4-H 2	20.2
		D 4-H 4	74.2	D 4-H 4	115	D 4-H 4	*
		D 4-H 6	94.3	D 4-H 6	107	D 4-H 6	*
		D 4-H 8	64.4	D 4-H 8	77.9	D 4-H 8	26.0
		D 4-H 12	48.0	D 4-H 12	44.2	D 4-H 12	23.4
		D 5-H 0	27.8	D 5-H 0	31.4	D 5-H 0	12.1
		D 6-H 0	32.3	D 6-H 0	31.6	D 6-H 0	15.8
		D 7-H 0	56.4	D 7-H 0	27.8	D 7-H 0	15.4
		D 7-H 2	45.2	D 7-H 2	78.8	D 7-H 2	23.9
		D 7-H 4	158	D 7-H 4	121	D 7-H 4	68.7
		D 7-H 6	153	D 7-H 6	99.7	D 7-H 6	32.9
		D 7-H 8	41.9	D 7-H 8	67.9	D 7-H 8	28.7
		D 7-H 12	78.4	D 7-H 12	53.0	D 7-H 12	17.7
		D 8-H 0	41.0	D 8-H 0	46.6	D 8-H 0	20.5
		D 9-H 0	35.8	D 9-H 0	32.1	D 9-H 0	20.0
		D 10-H 0	41.2	D 10-H 0	45.2	D 10-H 0	17.9
		D 11-H 0	37.1	D 11-H 0	37.7	D 11-H 0	17.9
		D 12-H 0	43.4	D 12-H 0	44.1	D 12-H 0	14.7
		D 13-H 0	37.1	D 13-H 0	37.7	D 13-H 0	23.0
		D 14-H 0	36.8	D 14-H 0	96.0	D 14-H 0	22.0
		D 14-H 2	102	D 15-H 0	35.7	D 14-H 2	20.5
		D 14-H 4	107	D 15-H 2	68.9	D 14-H 4	36.9
		D 14-H 6	95.6	D 15-H 4	131	D 14-H 6	29.0
		D 14-H 8	67.1	D 15-H 6	120	D 14-H 8	42.1
		D 14-H 12	62.6	D 15-H 8	103	D 14-H 12	30.8

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Final Data Hal/P 95-4 Chiral

17		18		19		20	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
		D 15-H 0	40.9	D 15-H 12	65.7	D 15-H 0	17.4
		D 16-H 0	47.6	D 16-H 0	60.3	D 16-H 0	19.0
		D 17-H 0	44.7	D 17-H 0	36.8	D 17-H 0	17.5
		D 18-H 0	38.6	D 18-H 0	45.0	D 18-H 0	19.8
		D 19-H 0	35.4	D 19-H 0	45.6	D 19-H 0	*
		D 20-H 0	33.9	D 20-H 0	59.5	D 20-H 0	23.2
		D 21-H 0	36.8	D 21-H 0	66.8	D 21-H 0	20.4
		D 21-H 2	57.9	D 21-H 2	41.9	D 21-H 2	33.8
		D 21-H 4	82.6	D 21-H 4	88.8	D 21-H 4	59.7
		D 21-H 6	102	D 21-H 6	73.7	D 21-H 6	34.8
		D 21-H 8	83.1	D 21-H 8	59.4	D 21-H 8	37.3
		D 21-H 12	52.7	D 21-H 12	32.9	D 21-H 12	30.4
		D 22-H 0	37.1	D 22-H 0	22.6	D 22-H 0	13.1
		D 25-H 0	24.7	D 25-H 0	17.8	D 25-H 0	24.6
		D 29-H 0	32.7	D 29-H 0	24.9	D 29-H 0	57.1
		D 32-H 0	87.4	D 32-H 0	27.1	D 30-H 0	51.6
		D 33-H 0	NS	D 33-H 0	33.7	D 32-H 0	26.0
		D 36-H 0	NS	D 36-H 0	32.4	D 33-H 0	22.5
		D 39-H 0	NS	D 39-H 0	35.5	D 35-H 0	18.1
		D 42-H 0	112	D 42-H 0	30.0	D 39-H 0	14.4
		D 42-H 0.5	127	D 42-H 0.5	29.0	D 42-H 0	17.0
		D 42-H 1	117	D 42-H 1	38.1	D 42-H 0.5	17.2
		D 42-H 2	159	D 42-H 2	55.0	D 42-H 1	24.8
		D 42-H 3	157	D 42-H 3	77.0	D 42-H 2	26.4
		D 42-H 4	204	D 42-H 4	70.3	D 42-H 3	57.5
		D 42-H 6	198	D 42-H 6	87.6	D 42-H 4	50.2
		D 42-H 8	166	D 42-H 8	63.5	D 42-H 6	53.4
		D 42-H 10	133	D 42-H 10	51.4	D 42-H 8	42.8
		D 42-H 12	128	D 42-H 12	46.3	D 42-H 10	37.3
		D 43-H 0	80.0	D 43-H 0	35.8	D 42-H 12	37.7
		D 44-H 0	NS	D 44-H 0	22.1	D 43-H 0	22.3
		D 45-H 0	47.3	D 45-H 0	17.6	D 44-H 0	14.2
		D 48-H 0	20.7	D 48-H 0	11.8	D 45-H 0	*
		D 52-H 0	21.7	D 51-H 0	10.5	D 48-H 0	*
		D 54-H 0	20.2	D 54-H 0	10.2	D 51-H 0	*
		D 57-H 0	17.3	D 57-H 0	11.0	D 60-H 0	*
		D 72-H 0	14.0	D 72-H 0	*	D 72-H 0	*
		D 180-H 0	NS	D 180-H 0	*	D 180-H 0	*

Final Data Hal/P 95-4 Chiral

5/4/98

001 (JKS)		002 (BSH)		04 (WPS)		005 (SGA)		007 (DAN)		008 (AYB)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*
D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*
D 1-H 3	*	D 1-H 3	*	D 1-H 3	*	D 1-H 3	*	D 1-H 3	*	D 1-H 3	*
D 1-H 4	*	D 1-H 4	*	D 1-H 4	*	D 1-H 4	*	D 1-H 4	*	D 1-H 4	16.4
D 1-H 6	*	D 1-H 6	*	D 1-H 6	20.9	D 1-H 6	*	D 1-H 6	*	D 1-H 6	18.8
D 1-H 8	*	D 1-H 8	*	D 1-H 8	18.5	D 1-H 8	*	D 1-H 8	*	D 1-H 8	21.5
D 1-H 10	*	D 1-H 10	*	D 1-H 10	*	D 1-H 10	*	D 1-H 10	*	D 1-H 10	*
D 1-H 12	*	D 1-H 12	*	D 1-H 12	*	D 1-H 12	*	D 1-H 12	*	D 1-H 12	*
D 2-H 0	*	D 2-H 0	*	D 2-H 0	18.0	D 2-H 0	*	D 2-H 0	*	D 2-H 0	*
D 3-H 0	*	D 3-H 0	*	D 3-H 0	20.1	D 3-H 0	*	D 3-H 0	*	D 3-H 0	*
D 4-H 0	*	D 4-H 0	*	D 4-H 0	30.3	D 4-H 0	*	D 4-H 0	22.6	D 4-H 0	17.3
D 4-H 2	*	D 4-H 2	*	D 4-H 2	62.0	D 4-H 2	*	D 4-H 2	34.6	D 4-H 2	26.0
D 4-H 4	*	D 4-H 4	19.0	D 4-H 4	40.5	D 4-H 4	*	D 4-H 4	*	D 4-H 4	47.2
D 4-H 6	*	D 4-H 6	*	D 4-H 6	54.8	D 4-H 6	*	D 4-H 6	50.3	D 4-H 6	27.2
D 4-H 8	*	D 4-H 8	*	D 4-H 8	55.3	D 4-H 8	22.4	D 4-H 8	40.2	D 4-H 8	33.9
D 4-H 12	*	D 4-H 12	*	D 4-H 12	51.5	D 4-H 10	20.7	D 4-H 12	39.3	D 4-H 12	37.8
D 5-H 0	17.2	D 5-H 0	*	D 5-H 0	65.8	D 5-H 0	28.8	D 5-H 0	22.9	D 5-H 0	26.9
D 6-H 0	20.8	D 6-H 0	*	D 6-H 0	53.7	D 6-H 0	16.2	D 6-H 0	31.7	D 6-H 0	31.8
D 7-H 0	26.1	D 7-H 0	18.0	D 7-H 0	52.1	D 7-H 0	17.9	D 7-H 0	80.9	D 7-H 0	46.0
D 7-H 2	16.8	D 7-H 2	16.8	D 7-H 2	45.9	D 7-H 2	15.6	D 7-H 2	40.9	D 7-H 2	46.3
D 7-H 4	32.6	D 7-H 4	29.7	D 7-H 4	64.7	D 7-H 4	26.0	D 7-H 4	28.6	D 7-H 4	61.4
D 7-H 6	37.2	D 7-H 6	17.6	D 7-H 6	44.6	D 7-H 6	26.0	D 7-H 6	40.6	D 7-H 6	74.1
D 7-H 8	74.0	D 7-H 8	23.3	D 7-H 8	32.2	D 7-H 8	18.7	D 7-H 8	42.8	D 7-H 8	59.9
D 7-H 12	56.4	D 7-H 12	29.3	D 7-H 12	28.2	D 7-H 12	*	D 7-H 12	42.4	D 7-H 12	54.2
D 8-H 0	62.7	D 8-H 0	20.2	D 8-H 0	20.1	D 8-H 0	17.9	D 8-H 0	24.8	D 8-H 0	38.4
D 9-H 0	72.6	D 9-H 0	23.9	D 9-H 0	24.4	D 9-H 0	27.4	D 9-H 0	21.3	D 9-H 0	59.0
D 10-H 0	87.8	D 10-H 0	31.1	D 10-H 0	26.3	D 10-H 0	23.2	D 10-H 0	27.0	D 10-H 0	65.0
D 11-H 0	54.2	D 11-H 0	34.3	D 11-H 0	30.3	D 11-H 0	22.1	D 11-H 0	33.9	D 11-H 0	NS
D 12-H 0	45.3	D 12-H 0	36.6	D 12-H 0	21.5	D 12-H 0	19.8	D 12-H 0	25.7	D 12-H 0	NS
D 13-H 0	44.1	D 13-H 0	36.0	D 13-H 0	23.9	D 13-H 0	26.6	D 13-H 0	27.9	D 13-H 0	NS
D 14-H 0	29.7	D 14-H 0	32.9	D 14-H 0	31.4	D 14-H 0	32.2	D 14-H 0	27.9	D 14-H 0	NS
D 14-H 2	22.8	D 14-H 2	29.7	D 14-H 2	32.5	D 14-H 2	19.8	D 14-H 2	27.0	D 14-H 2	NS
D 14-H 4	*	D 14-H 4	36.0	D 14-H 4	33.0	D 14-H 4	55.4	D 14-H 4	32.0	D 14-H 4	NS
D 14-H 6	36.4	D 14-H 6	39.8	D 14-H 6	39.5	D 14-H 6	36.1	D 14-H 6	41.5	D 14-H 6	NS
D 14-H 8	32.2	D 14-H 8	42.8	D 14-H 8	27.9	D 14-H 8	29.4	D 14-H 8	33.0	D 14-H 8	NS
D 14-H 12	35.0	D 14-H 12	36.0	D 14-H 12	22.5	D 14-H 12	29.1	D 14-H 12	43.7	D 14-H 12	NS

001 (JKS)		002 (BSH)		04 (WPS)		005 (SGA)		007 (DAN)		008 (AYB)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 15-H 0	41.3	D 15-H 0	29.7	D 15-H 0	28.2	D 15-H 0	42.5	D 15-H 0	40.6	D 15-H 0	NS
D 16-H 0	27.5	D 16-H 0	21.7	D 16-H 0	25.2	D 16-H 0	27.4	D 16-H 0	49.1	D 16-H 0	NS
D 17-H 0	31.1	D 17-H 0	30.1	D 17-H 0	31.7	D 17-H 0	23.5	D 17-H 0	37.1	D 17-H 0	NS
D 18-H 0	34.2	D 18-H 0	37.0	D 18-H 0	41.6	D 18-H 0	46.7	D 18-H 0	58.5	D 18-H 0	NS
D 19-H 0	41.1	D 19-H 0	39.4	D 19-H 0	40.8	D 19-H 0	29.1	D 19-H 0	32.7	D 19-H 0	NS
D 20-H 0	32.6	D 20-H 0	39.6	D 20-H 0	46.7	D 20-H 0	30.5	D 20-H 0	36.1	D 20-H 0	NS
D 21-H 0	38.8	D 21-H 0	33.9	D 21-H 0	45.4	D 21-H 0	39.7	D 21-H 0	54.8	D 21-H 0	NS
D 21-H 2	59.1	D 21-H 2	36.6	D 21-H 2	67.4	D 21-H 2	31.9	D 21-H 2	52.5	D 21-H 2	NS
D 21-H 4	51.0	D 21-H 4	43.4	D 21-H 4	41.1	D 21-H 4	32.2	D 21-H 4	61.7	D 21-H 4	NS
D 21-H 6	59.1	D 21-H 6	52.3	D 21-H 6	45.1	D 21-H 6	48.4	D 21-H 6	62.0	D 21-H 6	NS
D 21-H 8	32.2	D 21-H 8	59.7	D 21-H 8	67.1	D 21-H 8	31.9	D 21-H 8	48.4	D 21-H 8	NS
D 21-H 12	30.5	D 21-H 12	62.0	D 21-H 12	57.2	D 21-H 12	47.3	D 21-H 12	50.3	D 21-H 12	NS
D 22-H 0	38.2	D 22-H 0	35.6	D 22-H 0	43.8	D 22-H 0	24.9	D 22-H 0	41.8	D 22-H 0	NS
D 25-H 0	54.4	D 25-H 0	53.3	D 25-H 0	59.3	D 25-H 0	29.4	D 25-H 0	42.4	D 25-H 0	NS
D 29-H 0	51.2	D 29-H 0	48.9	D 29-H 0	60.4	D 29-H 0	NS	D 29-H 0	38.7	D 29-H 0	NS
D 32-H 0	33.6	D 32-H 0	74.9	D 32-H 0	54.0	D 32-H 0	NS	D 32-H 0	51.6	D 32-H 0	NS
D 33-H 0	49.0	D 34-H 0	57.9	D 33-H 0	NS	D 33-H 0	NS	D 35-H 0	34.9	D 33-H 0	NS
D 36-H 0	29.3	D 35-H 0	60.8	D 36-H 0	45.4	D 36-H 0	NS	D 36-H 0	47.5	D 36-H 0	NS
D 39-H 0	30.9	D 39-H 0	NS	D 37-H 0	31.4	D 39-H 0	NS	D 39-H 0	61.1	D 39-H 0	NS
D 42-H 0	52.8	D 42-H 0	NS	D 42-H 0	NS	D 42-H 0	NS	D 42-H 0	79.7	D 42-H 0	NS
D 42-H 0.5	63.5	D 42-H 0.5	NS	D 42-H 0.5	NS	D 42-H 0.5	NS	D 42-H 0.5	61.1	D 42-H 0.5	NS
D 42-H 1	53.0	D 42-H 1	NS	D 42-H 1	NS	D 42-H 1	NS	D 42-H 1	63.3	D 42-H 1	NS
D 42-H 2	67.0	D 42-H 2	NS	D 42-H 2	NS	D 42-H 2	NS	D 42-H 2	55.4	D 42-H 2	NS
D 42-H 3	66.8	D 42-H 3	NS	D 42-H 3	NS	D 42-H 3	NS	D 42-H 3	47.8	D 42-H 3	NS
D 42-H 4	53.8	D 42-H 4	NS	D 42-H 4	NS	D 42-H 4	NS	D 42-H 4	59.8	D 42-H 4	NS
D 42-H 6	59.1	D 42-H 6	NS	D 42-H 6	NS	D 42-H 6	NS	D 42-H 6	47.2	D 42-H 6	NS
D 42-H 8	61.1	D 42-H 8	NS	D 42-H 8	NS	D 42-H 8	NS	D 42-H 8	63.3	D 42-H 8	NS
D 42-H 10	32.6	D 42-H 10	NS	D 42-H 10	NS	D 42-H 10	NS	D 42-H 10	56.3	D 42-H 10	NS
D 42-H 12	77.7	D 42-H 12	NS	D 42-H 12	NS	D 42-H 12	NS	D 42-H 12	53.8	D 42-H 12	NS
D 43-H 0	29.7	D 43-H 0	NS	D 43-H 0	NS	D 43-H 0	NS	D 43-H 0	60.1	D 43-H 0	NS
D 44-H 0	39.0	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS
D 45-H 0	37.8	D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	20.7	D 45-H 0	NS
D 48-H 0	15.8	D 48-H 0	NS	D 48-H 0	NS	D 48-H 0	NS	D 49-H 0	NS	D 48-H 0	NS
D 51-H 0	*	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	*	D 51-H 0	NS
D 54-H 0	*	D 54-H 0	NS	D 54-H 0	NS	D 54-H 0	*	D 54-H 0	*	D 54-H 0	NS
D 57-H 0	*	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	*	D 57-H 0	NS
D 72-H 0	27.7	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	*	D 72-H 0	NS
D 180-H 0	*	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	*	D 180-H 0	NS

Final Data Hal/P 95-4 Chiral

5/4/98

009 (GRL)		010 (EYJ)		011 (CE)		014 (DLS)		015 (DMK)		016(LW)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*
D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*
D 1-H 3	*	D 1-H 3	*	D 1-H 3	*	D 1-H 3	*	D 1-H 3	*	D 1-H 3	*
D 1-H 4	*	D 1-H 4	15.1	D 1-H 4	24.2	D 1-H 4	*	D 1-H 4	*	D 1-H 4	*
D 1-H 6	*	D 1-H 6	16.5	D 1-H 6	*	D 1-H 6	*	D 1-H 6	*	D 1-H 6	*
D 1-H 8	*	D 1-H 8	16.7	D 1-H 8	17.2	D 1-H 8	*	D 1-H 8	*	D 1-H 8	*
D 1-H 10	*	D 1-H 10	*	D 1-H 10	16.9	D 1-H 10	*	D 1-H 10	*	D 1-H 10	*
D 1-H 12	*	D 1-H 12	*	D 1-H 12	17.9	D 1-H 12	*	D 1-H 12	*	D 1-H 12	*
D 2-H 0	*	D 2-H 0	*	D 2-H 0	16.2	D 2-H 0	*	D 2-H 0	*	D 2-H 0	*
D 3-H 0	*	D 3-H 0	*	D 3-H 0	44.0	D 3-H 0	*	D 3-H 0	*	D 3-H 0	*
D 4-H 0	*	D 4-H 0	18.4	D 4-H 0	40.9	D 4-H 0	*	D 4-H 0	*	D 4-H 0	*
D 4-H 2	*	D 4-H 2	19.2	D 4-H 2	41.4	D 4-H 2	*	D 4-H 2	*	D 4-H 2	*
D 4-H 4	23.1	D 4-H 4	19.4	D 4-H 4	59.7	D 4-H 4	15.8	D 4-H 4	18.0	D 4-H 4	18.7
D 4-H 6	15.2	D 4-H 6	20.2	D 4-H 6	53.8	D 4-H 6	15.8	D 4-H 6	22.3	D 4-H 6	18.2
D 4-H 8	16.6	D 4-H 8	24.8	D 4-H 8	51.9	D 4-H 8	*	D 4-H 8	20.9	D 4-H 8	*
D 4-H 12	15.7	D 4-H 12	24.2	D 4-H 12	45.1	D 4-H 12	*	D 4-H 12	*	D 4-H 12	22.7
D 5-H 0	17.8	D 5-H 0	21.7	D 5-H 0	41.6	D 5-H 0	*	D 5-H 0	*	D 5-H 0	17.6
D 6-H 0	15.2	D 6-H 0	19.2	D 6-H 0	46.8	D 6-H 0	*	D 6-H 0	16.9	D 6-H 0	29.3
D 7-H 0	21.7	D 7-H 0	*	D 7-H 0	29.8	D 7-H 0	*	D 7-H 0	16.3	D 7-H 0	36.7
D 7-H 2	18.7	D 7-H 2	*	D 7-H 2	30.6	D 7-H 2	*	D 7-H 2	15.3	D 7-H 2	35.6
D 7-H 4	41.5	D 7-H 4	19.0	D 7-H 4	47.5	D 7-H 4	*	D 7-H 4	20.9	D 7-H 4	24.7
D 7-H 6	33.2	D 7-H 6	18.8	D 7-H 6	36.2	D 7-H 6	18.2	D 7-H 6	20.0	D 7-H 6	20.7
D 7-H 8	28.5	D 7-H 8	16.5	D 7-H 8	33.6	D 7-H 8	*	D 7-H 8	*	D 7-H 8	34.7
D 7-H 12	35.3	D 7-H 12	*	D 7-H 12	32.9	D 7-H 12	*	D 7-H 12	*	D 7-H 12	35.6
D 8-H 0	29.1	D 8-H 0	15.3	D 8-H 0	32.0	D 8-H 0	*	D 8-H 0	*	D 8-H 0	27.6
D 9-H 0	29.7	D 9-H 0	28.8	D 9-H 0	35.7	D 9-H 0	15.6	D 9-H 0	*	D 9-H 0	32.7
D 10-H 0	25.5	D 10-H 0	22.1	D 10-H 0	25.6	D 10-H 0	26.5	D 10-H 0	20.0	D 10-H 0	41.6
D 11-H 0	45.3	D 11-H 0	33.5	D 11-H 0	26.6	D 11-H 0	20.5	D 11-H 0	16.7	D 11-H 0	31.6
D 12-H 0	36.8	D 12-H 0	22.1	D 12-H 0	27.7	D 12-H 0	28.6	D 12-H 0	20.2	D 12-H 0	31.6
D 13-H 0	29.1	D 13-H 0	20.3	D 13-H 0	42.1	D 13-H 0	25.2	D 13-H 0	16.9	D 13-H 0	33.3
D 14-H 0	27.3	D 14-H 0	18.0	D 14-H 0	32.0	D 14-H 0	28.4	D 14-H 0	19.8	D 14-H 0	32.5
D 14-H 2	27.0	D 14-H 2	20.9	D 14-H 2	25.9	D 14-H 2	34.4	D 14-H 2	27.5	D 14-H 2	36.5
D 14-H 4	56.9	D 14-H 4	36.8	D 14-H 4	41.1	D 14-H 4	50.0	D 14-H 4	36.2	D 14-H 4	52.2
D 14-H 6	51.9	D 14-H 6	40.8	D 14-H 6	47.5	D 14-H 6	44.0	D 14-H 6	22.3	D 14-H 6	55.9
D 14-H 8	73.2	D 14-H 8	48.3	D 14-H 8	41.8	D 14-H 8	55.5	D 14-H 8	30.8	D 14-H 8	48.7
D 14-H 12	63.7	D 14-H 12	40.2	D 14-H 12	50.3	D 14-H 12	39.6	D 14-H 12	24.4	D 14-H 12	48.7

009 (GRL)		010 (EYJ)		011 (CE)		014 (DLS)		015 (DMK)		016(LW)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 15-H 0	35.9	D 15-H 0	31.9	D 15-H 0	42.8	D 15-H 0	38.8	D 15-H 0	23.3	D 15-H 0	41.3
D 16-H 0	43.9	D 16-H 0	28.1	D 16-H 0	56.6	D 16-H 0	38.8	D 16-H 0	24.2	D 16-H 0	39.9
D 17-H 0	48.9	D 17-H 0	36.0	D 17-H 0	41.6	D 17-H 0	40.4	D 17-H 0	31.8	D 17-H 0	44.7
D 18-H 0	56.6	D 18-H 0	34.1	D 18-H 0	34.8	D 18-H 0	35.4	D 18-H 0	35.8	D 18-H 0	46.5
D 19-H 0	34.4	D 19-H 0	45.3	D 19-H 0	44.4	D 19-H 0	35.2	D 19-H 0	21.3	D 19-H 0	49.6
D 20-H 0	42.1	D 20-H 0	41.4	D 20-H 0	33.6	D 20-H 0	31.2	D 20-H 0	21.3	D 20-H 0	51.9
D 21-H 0	31.7	D 21-H 0	33.5	D 21-H 0	27.7	D 21-H 0	20.0	D 21-H 0	*	D 21-H 0	43.3
D 21-H 2	39.1	D 21-H 2	43.7	D 21-H 2	31.3	D 21-H 2	37.8	D 21-H 2	20.7	D 21-H 2	43.9
D 21-H 4	53.3	D 21-H 4	47.4	D 21-H 4	27.0	D 21-H 4	31.2	D 21-H 4	31.8	D 21-H 4	45.0
D 21-H 6	47.7	D 21-H 6	55.5	D 21-H 6	39.3	D 21-H 6	29.9	D 21-H 6	22.3	D 21-H 6	45.6
D 21-H 8	66.4	D 21-H 8	61.1	D 21-H 8	32.7	D 21-H 8	23.1	D 21-H 8	31.2	D 21-H 8	68.7
D 21-H 12	59.8	D 21-H 12	44.7	D 21-H 12	39.3	D 21-H 12	22.1	D 21-H 12	20.9	D 21-H 12	51.6
D 22-H 0	54.5	D 22-H 0	38.5	D 22-H 0	27.5	D 22-H 0	29.4	D 22-H 0	28.7	D 22-H 0	37.6
D 25-H 0	45.6	D 25-H 0	46.4	D 25-H 0	32.9	D 25-H 0	32.8	D 25-H 0	22.9	D 25-H 0	44.2
D 29-H 0	42.4	D 29-H 0	36.0	D 29-H 0	28.9	D 29-H 0	56.3	D 29-H 0	42.0	D 29-H 0	34.7
D 32-H 0	42.4	D 32-H 0	43.5	D 32-H 0	35.7	D 32-H 0	38.0	D 32-H 0	42.0	D 32-H 0	41.6
D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS
D 36-H 0	30.2	D 36-H 0	57.4	D 36-H 0	NS	D 36-H 0	48.5	D 36-H 0	33.1	D 36-H 0	47.9
D 39-H 0	44.8	D 39-H 0	50.3	D 39-H 0	58.1	D 39-H 0	54.5	D 39-H 0	36.4	D 39-H 0	73.0
D 42-H 0	35.0	D 42-H 0	26.1	D 42-H 0	39.5	D 42-H 0	NS	D 42-H 0	*	D 42-H 0	28.2
D 42-H 0.5	19.9	D 42-H 0.5	31.7	D 42-H 0.5	39.7	D 42-H 0.5	NS	D 42-H 0.5	20.2	D 42-H 0.5	31.9
D 42-H 1	19.0	D 42-H 1	19.8	D 42-H 1	48.2	D 42-H 1	NS	D 42-H 1	31.2	D 42-H 1	32.2
D 42-H 2	30.8	D 42-H 2	54.9	D 42-H 2	66.1	D 42-H 2	NS	D 42-H 2	18.8	D 42-H 2	32.2
D 42-H 3	36.8	D 42-H 3	44.1	D 42-H 3	65.6	D 42-H 3	NS	D 42-H 3	52.3	D 42-H 3	47.9
D 42-H 4	19.3	D 42-H 4	48.7	D 42-H 4	74.0	D 42-H 4	NS	D 42-H 4	39.1	D 42-H 4	43.3
D 42-H 6	37.1	D 42-H 6	68.2	D 42-H 6	49.8	D 42-H 6	NS	D 42-H 6	19.6	D 42-H 6	28.2
D 42-H 8	35.0	D 42-H 8	47.4	D 42-H 8	51.9	D 42-H 8	NS	D 42-H 8	26.7	D 42-H 8	33.0
D 42-H 10	32.0	D 42-H 10	55.9	D 42-H 10	77.6	D 42-H 10	NS	D 42-H 10	29.1	D 42-H 10	42.7
D 42-H 12	32.3	D 42-H 12	56.3	D 42-H 12	46.8	D 42-H 12	NS	D 42-H 12	38.3	D 42-H 12	33.3
D 43-H 0	30.5	D 43-H 0	60.3	D 43-H 0	45.4	D 43-H 0	NS	D 43-H 0	34.3	D 43-H 0	28.5
D 44-H 0	28.2	D 44-H 0	31.5	D 44-H 0	35.7	D 44-H 0	NS	D 44-H 0	20.9	D 44-H 0	41.0
D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	24.4	D 45-H 0	NS	D 45-H 0	17.3	D 45-H 0	NS
D 48-H 0	*	D 49-H 0	*	D 48-H 0	NS	D 48-H 0	NS	D 48-H 0	*	D 48-H 0	NS
D 51-H 0	*	D 51-H 0	*	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	*	D 51-H 0	NS
D 54-H 0	*	D 54-H 0	*	D 54-H 0	NS	D 54-H 0	NS	D 54-H 0	*	D 54-H 0	NS
D 57-H 0	*	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	*	D 57-H 0	NS
D 72-H 0	*	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	*	D 76-H 0	*
D 180-H 0	*	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	*	D 180-H 0	*

Final Data Hal/P 95-4 Chiral

17		18		19		20	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*
D 1-H 2	*	D 1-H 2	28.4	D 1-H 2	18.4	D 1-H 2	*
D 1-H 3	*	D 1-H 3	16.5	D 1-H 3	15.8	D 1-H 3	*
D 1-H 4	*	D 1-H 4	15.5	D 1-H 4	*	D 1-H 4	*
D 1-H 6	*	D 1-H 6	16.3	D 1-H 6	*	D 1-H 6	*
		D 1-H 8	18.5	D 1-H 8	*	D 1-H 8	*
		D 1-H 10	15.5	D 1-H 10	*	D 1-H 10	*
		D 1-H 12	16.3	D 1-H 12	*	D 1-H 12	*
		D 2-H 0	20.4	D 2-H 0	22.0	D 2-H 0	47.5
		D 3-H 0	*	D 3-H 0	*	D 3-H 0	*
		D 4-H 0	23.4	D 4-H 0	15.5	D 4-H 0	23.6
		D 4-H 2	19.5	D 4-H 2	23.1	D 4-H 2	24.0
		D 4-H 4	25.4	D 4-H 4	23.6	D 4-H 4	*
		D 4-H 6	23.2	D 4-H 6	*	D 4-H 6	*
		D 4-H 8	19.5	D 4-H 8	23.1	D 4-H 8	32.3
		D 4-H 12	18.9	D 4-H 12	*	D 4-H 12	26.0
		D 5-H 0	25.6	D 5-H 0	*	D 5-H 0	23.1
		D 6-H 0	31.2	D 6-H 0	15.5	D 6-H 0	28.7
		D 7-H 0	27.2	D 7-H 0	15.8	D 7-H 0	22.1
		D 7-H 2	26.0	D 7-H 2	*	D 7-H 2	29.1
		D 7-H 4	33.4	D 7-H 4	17.2	D 7-H 4	181
		D 7-H 6	28.2	D 7-H 6	*	D 7-H 6	28.5
		D 7-H 8	24.8	D 7-H 8	*	D 7-H 8	31.4
		D 7-H 12	26.0	D 7-H 12	*	D 7-H 12	25.8
		D 8-H 0	28.6	D 8-H 0	15.3	D 8-H 0	28.7
		D 9-H 0	27.0	D 9-H 0	15.5	D 9-H 0	29.4
		D 10-H 0	32.8	D 10-H 0	27.7	D 10-H 0	26.0
		D 11-H 0	39.0	D 11-H 0	20.5	D 11-H 0	31.8
		D 12-H 0	38.2	D 12-H 0	25.3	D 12-H 0	29.4
		D 13-H 0	26.4	D 13-H 0	22.2	D 13-H 0	34.1
		D 14-H 0	28.4	D 14-H 0	33.4	D 14-H 0	26.9
		D 14-H 2	35.0	D 15-H 0	23.9	D 14-H 2	22.5
		D 14-H 4	37.6	D 15-H 2	26.0	D 14-H 4	40.7
		D 14-H 6	30.2	D 15-H 4	17.7	D 14-H 6	37.0
		D 14-H 8	26.4	D 15-H 6	18.9	D 14-H 8	36.0
		D 14-H 12	33.8	D 15-H 8	24.8	D 14-H 12	26.5

17		18		19		20	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
		D 15-H 0	22.2	D 15-H 12	*	D 15-H 0	25.6
		D 16-H 0	30.0	D 16-H 0	21.0	D 16-H 0	30.0
		D 17-H 0	24.2	D 17-H 0	22.4	D 17-H 0	30.4
		D 18-H 0	26.4	D 18-H 0	28.4	D 18-H 0	41.8
		D 19-H 0	28.4	D 19-H 0	*	D 19-H 0	*
		D 20-H 0	28.2	D 20-H 0	*	D 20-H 0	21.1
		D 21-H 0	27.8	D 21-H 0	20.1	D 21-H 0	21.9
		D 21-H 2	31.8	D 21-H 2	*	D 21-H 2	27.9
		D 21-H 4	23.4	D 21-H 4	*	D 21-H 4	33.3
		D 21-H 6	31.4	D 21-H 6	26.2	D 21-H 6	26.5
		D 21-H 8	30.8	D 21-H 8	34.3	D 21-H 8	25.6
		D 21-H 12	23.2	D 21-H 12	17.0	D 21-H 12	31.0
		D 22-H 0	27.6	D 22-H 0	19.8	D 22-H 0	20.7
		D 25-H 0	17.7	D 25-H 0	*	D 25-H 0	18.6
		D 29-H 0	19.7	D 29-H 0	17.4	D 29-H 0	39.1
		D 32-H 0	29.8	D 32-H 0	18.9	D 30-H 0	44.5
		D 33-H 0	NS	D 33-H 0	21.7	D 32-H 0	49.0
		D 36-H 0	NS	D 36-H 0	21.2	D 33-H 0	42.0
		D 39-H 0	NS	D 39-H 0	29.3	D 35-H 0	53.1
		D 42-H 0	39.2	D 42-H 0	*	D 39-H 0	43.0
		D 42-H 0.5	37.8	D 42-H 0.5	*	D 42-H 0	28.5
		D 42-H 1	37.8	D 42-H 1	21.7	D 42-H 0.5	33.0
		D 42-H 2	38.4	D 42-H 2	19.3	D 42-H 1	40.9
		D 42-H 3	33.8	D 42-H 3	28.1	D 42-H 2	38.7
		D 42-H 4	33.6	D 42-H 4	23.9	D 42-H 3	49.3
		D 42-H 6	43.3	D 42-H 6	33.1	D 42-H 4	45.9
		D 42-H 8	37.8	D 42-H 8	33.8	D 42-H 6	41.1
		D 42-H 10	45.3	D 42-H 10	28.4	D 42-H 8	45.9
		D 42-H 12	32.8	D 42-H 12	23.1	D 42-H 10	42.7
		D 43-H 0	31.8	D 43-H 0	19.1	D 42-H 12	41.6
		D 44-H 0	NS	D 44-H 0	16.7	D 43-H 0	33.2
		D 45-H 0	18.1	D 45-H 0	18.6	D 44-H 0	31.4
		D 48-H 0	17.7	D 48-H 0	*	D 45-H 0	24.2
		D 52-H 0	*	D 51-H 0	*	D 48-H 0	*
		D 54-H 0	*	D 54-H 0	*	D 51-H 0	*
		D 57-H 0	*	D 57-H 0	*	D 60-H 0	34.8
		D 72-H 0	*	D 72-H 0	*	D 72-H 0	31.9
		D 180-H 0	NS	D 180-H 0	*	D 180-H 0	29.4

Final Data Hal/P 95-4 Chiral

5/4/98

001 (JKS)		002 (BSH)		04 (WPS)		005 (SGA)		007 (DAN)		008 (AYB)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*
D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*
D 1-H 3	16.4	D 1-H 3	*	D 1-H 3	18.6	D 1-H 3	15.0	D 1-H 3	*	D 1-H 3	33.1
D 1-H 4	20.9	D 1-H 4	*	D 1-H 4	20.1	D 1-H 4	15.0	D 1-H 4	15.8	D 1-H 4	35.7
D 1-H 6	18.5	D 1-H 6	*	D 1-H 6	46.0	D 1-H 6	29.3	D 1-H 6	15.8	D 1-H 6	50.5
D 1-H 8	27.0	D 1-H 8	*	D 1-H 8	47.1	D 1-H 8	18.7	D 1-H 8	17.6	D 1-H 8	51.5
D 1-H 10	27.7	D 1-H 10	*	D 1-H 10	33.9	D 1-H 10	47.3	D 1-H 10	18.3	D 1-H 10	37.1
D 1-H 12	22.3	D 1-H 12	*	D 1-H 12	40.7	D 1-H 12	18.4	D 1-H 12	*	D 1-H 12	41.4
D 2-H 0	23.2	D 2-H 0	*	D 2-H 0	38.0	D 2-H 0	29.3	D 2-H 0	*	D 2-H 0	33.4
D 3-H 0	29.5	D 3-H 0	*	D 3-H 0	69.5	D 3-H 0	54.2	D 3-H 0	38.9	D 3-H 0	44.1
D 4-H 0	36.4	D 4-H 0	31.5	D 4-H 0	96.8	D 4-H 0	46.4	D 4-H 0	71.8	D 4-H 0	76.5
D 4-H 2	38.4	D 4-H 2	60.6	D 4-H 2	199	D 4-H 2	40.5	D 4-H 2	92.8	D 4-H 2	100
D 4-H 4	44.3	D 4-H 4	61.2	D 4-H 4	125	D 4-H 4	88.0	D 4-H 4	110	D 4-H 4	199
D 4-H 6	84.7	D 4-H 6	63.6	D 4-H 6	165	D 4-H 6	70.0	D 4-H 6	135	D 4-H 6	110
D 4-H 8	52.4	D 4-H 8	34.7	D 4-H 8	192	D 4-H 8	98.6	D 4-H 8	131	D 4-H 8	129
D 4-H 12	64.7	D 4-H 12	53.0	D 4-H 12	181	D 4-H 10	97.0	D 4-H 12	101	D 4-H 12	132
D 5-H 0	76.2	D 5-H 0	55.8	D 5-H 0	211	D 5-H 0	118	D 5-H 0	93.8	D 5-H 0	115
D 6-H 0	108	D 6-H 0	77.7	D 6-H 0	234	D 6-H 0	100	D 6-H 0	132	D 6-H 0	139
D 7-H 0	128	D 7-H 0	84.4	D 7-H 0	260	D 7-H 0	136	D 7-H 0	211	D 7-H 0	245
D 7-H 2	74.1	D 7-H 2	71.8	D 7-H 2	242	D 7-H 2	79.3	D 7-H 2	202	D 7-H 2	205
D 7-H 4	153	D 7-H 4	121	D 7-H 4	314	D 7-H 4	175	D 7-H 4	106	D 7-H 4	310
D 7-H 6	202	D 7-H 6	78.1	D 7-H 6	207	D 7-H 6	199	D 7-H 6	150	D 7-H 6	351
D 7-H 8	203	D 7-H 8	91.8	D 7-H 8	149	D 7-H 8	139	D 7-H 8	135	D 7-H 8	296
D 7-H 12	149	D 7-H 12	100	D 7-H 12	134	D 7-H 12	83.0	D 7-H 12	138	D 7-H 12	286
D 8-H 0	183	D 8-H 0	93.8	D 8-H 0	92.4	D 8-H 0	131	D 8-H 0	113	D 8-H 0	211
D 9-H 0	221	D 9-H 0	101	D 9-H 0	138	D 9-H 0	179	D 9-H 0	102	D 9-H 0	283
D 10-H 0	282	D 10-H 0	140	D 10-H 0	138	D 10-H 0	155	D 10-H 0	132	D 10-H 0	363
D 11-H 0	227	D 11-H 0	174	D 11-H 0	175	D 11-H 0	161	D 11-H 0	138	D 11-H 0	NS
D 12-H 0	325	D 12-H 0	174	D 12-H 0	135	D 12-H 0	186	D 12-H 0	123	D 12-H 0	NS
D 13-H 0	334	D 13-H 0	194	D 13-H 0	121	D 13-H 0	283	D 13-H 0	128	D 13-H 0	NS
D 14-H 0	311	D 14-H 0	161	D 14-H 0	200	D 14-H 0	304	D 14-H 0	174	D 14-H 0	NS
D 14-H 2	211	D 14-H 2	160	D 14-H 2	160	D 14-H 2	192	D 14-H 2	163	D 14-H 2	NS
D 14-H 4	*	D 14-H 4	188	D 14-H 4	174	D 14-H 4	453	D 14-H 4	138	D 14-H 4	NS
D 14-H 6	324	D 14-H 6	229	D 14-H 6	193	D 14-H 6	274	D 14-H 6	180	D 14-H 6	NS
D 14-H 8	318	D 14-H 8	232	D 14-H 8	124	D 14-H 8	235	D 14-H 8	143	D 14-H 8	NS
D 14-H 12	260	D 14-H 12	201	D 14-H 12	114	D 14-H 12	198	D 14-H 12	187	D 14-H 12	NS

001 (JKS)		002 (BSH)		04 (WPS)		005 (SGA)		007 (DAN)		008 (AYB)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 15-H 0	320	D 15-H 0	154	D 15-H 0	169	D 15-H 0	355	D 15-H 0	212	D 15-H 0	NS
D 16-H 0	296	D 16-H 0	147	D 16-H 0	171	D 16-H 0	298	D 16-H 0	214	D 16-H 0	NS
D 17-H 0	300	D 17-H 0	200	D 17-H 0	214	D 17-H 0	214	D 17-H 0	142	D 17-H 0	NS
D 18-H 0	351	D 18-H 0	214	D 18-H 0	243	D 18-H 0	390	D 18-H 0	266	D 18-H 0	NS
D 19-H 0	409	D 19-H 0	218	D 19-H 0	264	D 19-H 0	297	D 19-H 0	166	D 19-H 0	NS
D 20-H 0	361	D 20-H 0	219	D 20-H 0	313	D 20-H 0	331	D 20-H 0	174	D 20-H 0	NS
D 21-H 0	497	D 21-H 0	193	D 21-H 0	283	D 21-H 0	340	D 21-H 0	257	D 21-H 0	NS
D 21-H 2	532	D 21-H 2	209	D 21-H 2	449	D 21-H 2	333	D 21-H 2	246	D 21-H 2	NS
D 21-H 4	435	D 21-H 4	263	D 21-H 4	303	D 21-H 4	312	D 21-H 4	279	D 21-H 4	NS
D 21-H 6	496	D 21-H 6	274	D 21-H 6	285	D 21-H 6	438	D 21-H 6	260	D 21-H 6	NS
D 21-H 8	290	D 21-H 8	336	D 21-H 8	368	D 21-H 8	260	D 21-H 8	211	D 21-H 8	NS
D 21-H 12	251	D 21-H 12	372	D 21-H 12	330	D 21-H 12	401	D 21-H 12	212	D 21-H 12	NS
D 22-H 0	295	D 22-H 0	243	D 22-H 0	296	D 22-H 0	246	D 22-H 0	205	D 22-H 0	NS
D 25-H 0	386	D 25-H 0	289	D 25-H 0	349	D 25-H 0	32.1	D 25-H 0	184	D 25-H 0	NS
D 29-H 0	335	D 29-H 0	292	D 29-H 0	318	D 29-H 0	NS	D 29-H 0	177	D 29-H 0	NS
D 32-H 0	385	D 32-H 0	432	D 32-H 0	411	D 32-H 0	NS	D 32-H 0	297	D 32-H 0	NS
D 33-H 0	329	D 34-H 0	431	D 33-H 0	NS	D 33-H 0	NS	D 35-H 0	189	D 33-H 0	NS
D 36-H 0	289	D 35-H 0	459	D 36-H 0	259	D 36-H 0	NS	D 36-H 0	267	D 36-H 0	NS
D 39-H 0	355	D 39-H 0	NS	D 37-H 0	219	D 39-H 0	NS	D 39-H 0	312	D 39-H 0	NS
D 42-H 0	484	D 42-H 0	NS	D 42-H 0	NS	D 42-H 0	NS	D 42-H 0	402	D 42-H 0	NS
D 42-H 0.5	506	D 42-H 0.5	NS	D 42-H 0.5	NS	D 42-H 0.5	NS	D 42-H 0.5	246	D 42-H 0.5	NS
D 42-H 1	468	D 42-H 1	NS	D 42-H 1	NS	D 42-H 1	NS	D 42-H 1	289	D 42-H 1	NS
D 42-H 2	535	D 42-H 2	NS	D 42-H 2	NS	D 42-H 2	NS	D 42-H 2	244	D 42-H 2	NS
D 42-H 3	687	D 42-H 3	NS	D 42-H 3	NS	D 42-H 3	NS	D 42-H 3	277	D 42-H 3	NS
D 42-H 4	565	D 42-H 4	NS	D 42-H 4	NS	D 42-H 4	NS	D 42-H 4	382	D 42-H 4	NS
D 42-H 6	576	D 42-H 6	NS	D 42-H 6	NS	D 42-H 6	NS	D 42-H 6	350	D 42-H 6	NS
D 42-H 8	538	D 42-H 8	NS	D 42-H 8	NS	D 42-H 8	NS	D 42-H 8	408	D 42-H 8	NS
D 42-H 10	339	D 42-H 10	NS	D 42-H 10	NS	D 42-H 10	NS	D 42-H 10	335	D 42-H 10	NS
D 42-H 12	633	D 42-H 12	NS	D 42-H 12	NS	D 42-H 12	NS	D 42-H 12	353	D 42-H 12	NS
D 43-H 0	224	D 43-H 0	NS	D 43-H 0	NS	D 43-H 0	NS	D 43-H 0	382	D 43-H 0	NS
D 44-H 0	424	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS	D 44-H 0	NS
D 45-H 0	508	D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	217	D 45-H 0	NS
D 48-H 0	340	D 48-H 0	NS	D 48-H 0	NS	D 48-H 0	NS	D 49-H 0	NS	D 48-H 0	NS
D 51-H 0	255	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	97.3	D 51-H 0	NS
D 54-H 0	175	D 54-H 0	NS	D 54-H 0	NS	D 54-H 0	20.9	D 54-H 0	48.0	D 54-H 0	NS
D 57-H 0	128	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	51.5	D 57-H 0	NS
D 72-H 0	243	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	17.2	D 72-H 0	NS
D 180-H 0	16.4	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	*	D 180-H 0	NS

009 (GRL)		010 (EYJ)		011 (CE)		014 (DLS)		015 (DMK)		016(LW)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*
D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*
D 1-H 3	*	D 1-H 3	29.3	D 1-H 3	*	D 1-H 3	*	D 1-H 3	*	D 1-H 3	*
D 1-H 4	20.4	D 1-H 4	31.0	D 1-H 4	45.7	D 1-H 4	17.1	D 1-H 4	15.7	D 1-H 4	*
D 1-H 6	24.3	D 1-H 6	33.7	D 1-H 6	20.8	D 1-H 6	15.6	D 1-H 6	*	D 1-H 6	*
D 1-H 8	27.6	D 1-H 8	33.7	D 1-H 8	31.0	D 1-H 8	19.1	D 1-H 8	*	D 1-H 8	*
D 1-H 10	26.6	D 1-H 10	32.2	D 1-H 10	31.3	D 1-H 10	22.6	D 1-H 10	*	D 1-H 10	16.6
D 1-H 12	23.0	D 1-H 12	23.0	D 1-H 12	35.7	D 1-H 12	22.3	D 1-H 12	*	D 1-H 12	19.5
D 2-H 0	24.0	D 2-H 0	20.4	D 2-H 0	31.5	D 2-H 0	18.8	D 2-H 0	*	D 2-H 0	18.5
D 3-H 0	33.2	D 3-H 0	33.5	D 3-H 0	100	D 3-H 0	19.4	D 3-H 0	23.9	D 3-H 0	49.1
D 4-H 0	44.1	D 4-H 0	45.9	D 4-H 0	96.3	D 4-H 0	33.3	D 4-H 0	50.3	D 4-H 0	78.1
D 4-H 2	51.7	D 4-H 2	51.8	D 4-H 2	95.0	D 4-H 2	38.6	D 4-H 2	58.1	D 4-H 2	85.1
D 4-H 4	78.5	D 4-H 4	70.1	D 4-H 4	164	D 4-H 4	59.8	D 4-H 4	88.6	D 4-H 4	132
D 4-H 6	64.3	D 4-H 6	66.8	D 4-H 6	137	D 4-H 6	59.5	D 4-H 6	108	D 4-H 6	99.5
D 4-H 8	55.0	D 4-H 8	73.1	D 4-H 8	137	D 4-H 8	33.6	D 4-H 8	89.7	D 4-H 8	77.5
D 4-H 12	44.1	D 4-H 12	68.7	D 4-H 12	125	D 4-H 12	45.8	D 4-H 12	68.8	D 4-H 12	128
D 5-H 0	85.8	D 5-H 0	71.4	D 5-H 0	103	D 5-H 0	29.6	D 5-H 0	41.0	D 5-H 0	123
D 6-H 0	62.6	D 6-H 0	71.0	D 6-H 0	139	D 6-H 0	83.3	D 6-H 0	89.0	D 6-H 0	200
D 7-H 0	111	D 7-H 0	51.0	D 7-H 0	84.8	D 7-H 0	53.4	D 7-H 0	97.5	D 7-H 0	267
D 7-H 2	79.8	D 7-H 2	60.0	D 7-H 2	101	D 7-H 2	59.2	D 7-H 2	87.9	D 7-H 2	238
D 7-H 4	194	D 7-H 4	79.0	D 7-H 4	142	D 7-H 4	70.5	D 7-H 4	149	D 7-H 4	157
D 7-H 6	176	D 7-H 6	70.3	D 7-H 6	103	D 7-H 6	84.7	D 7-H 6	112	D 7-H 6	146
D 7-H 8	125	D 7-H 8	67.4	D 7-H 8	94.8	D 7-H 8	63.0	D 7-H 8	53.8	D 7-H 8	214
D 7-H 12	164	D 7-H 12	54.3	D 7-H 12	93.4	D 7-H 12	51.1	D 7-H 12	49.2	D 7-H 12	254
D 8-H 0	158	D 8-H 0	61.1	D 8-H 0	96.9	D 8-H 0	55.1	D 8-H 0	83.6	D 8-H 0	226
D 9-H 0	138	D 9-H 0	104	D 9-H 0	126	D 9-H 0	72.0	D 9-H 0	55.1	D 9-H 0	256
D 10-H 0	153	D 10-H 0	103	D 10-H 0	86.6	D 10-H 0	116	D 10-H 0	120	D 10-H 0	335
D 11-H 0	240	D 11-H 0	137	D 11-H 0	97.4	D 11-H 0	97.8	D 11-H 0	102	D 11-H 0	292
D 12-H 0	228	D 12-H 0	90.6	D 12-H 0	102	D 12-H 0	155	D 12-H 0	135	D 12-H 0	290
D 13-H 0	180	D 13-H 0	81.9	D 13-H 0	167	D 13-H 0	196	D 13-H 0	110	D 13-H 0	317
D 14-H 0	174	D 14-H 0	73.7	D 14-H 0	135	D 14-H 0	195	D 14-H 0	154	D 14-H 0	334
D 14-H 2	176	D 14-H 2	96.9	D 14-H 2	102	D 14-H 2	298	D 14-H 2	186	D 14-H 2	354
D 14-H 4	382	D 14-H 4	132	D 14-H 4	157	D 14-H 4	320	D 14-H 4	221	D 14-H 4	462
D 14-H 6	337	D 14-H 6	150	D 14-H 6	167	D 14-H 6	317	D 14-H 6	137	D 14-H 6	473
D 14-H 8	423	D 14-H 8	156	D 14-H 8	133	D 14-H 8	374	D 14-H 8	180	D 14-H 8	430
D 14-H 12	400	D 14-H 12	152	D 14-H 12	155	D 14-H 12	299	D 14-H 12	169	D 14-H 12	430

009 (GRL)		010 (EYJ)		011 (CE)		014 (DLS)		015 (DMK)		016(LW)	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 15-H 0	228	D 15-H 0	131	D 15-H 0	144	D 15-H 0	26.7	D 15-H 0	166	D 15-H 0	435
D 16-H 0	288	D 16-H 0	127	D 16-H 0	193	D 16-H 0	317	D 16-H 0	174	D 16-H 0	453
D 17-H 0	313	D 17-H 0	184	D 17-H 0	189	D 17-H 0	309	D 17-H 0	200	D 17-H 0	459
D 18-H 0	378	D 18-H 0	154	D 18-H 0	143	D 18-H 0	292	D 18-H 0	234	D 18-H 0	513
D 19-H 0	252	D 19-H 0	207	D 19-H 0	193	D 19-H 0	320	D 19-H 0	167	D 19-H 0	561
D 20-H 0	289	D 20-H 0	202	D 20-H 0	137	D 20-H 0	303	D 20-H 0	188	D 20-H 0	588
D 21-H 0	264	D 21-H 0	200	D 21-H 0	130	D 21-H 0	199	D 21-H 0	137	D 21-H 0	521
D 21-H 2	300	D 21-H 2	245	D 21-H 2	118	D 21-H 2	336	D 21-H 2	180	D 21-H 2	587
D 21-H 4	386	D 21-H 4	209	D 21-H 4	98.7	D 21-H 4	233	D 21-H 4	250	D 21-H 4	548
D 21-H 6	312	D 21-H 6	213	D 21-H 6	195	D 21-H 6	244	D 21-H 6	173	D 21-H 6	532
D 21-H 8	384	D 21-H 8	255	D 21-H 8	131	D 21-H 8	176	D 21-H 8	251	D 21-H 8	715
D 21-H 12	386	D 21-H 12	200	D 21-H 12	176	D 21-H 12	161	D 21-H 12	178	D 21-H 12	492
D 22-H 0	393	D 22-H 0	212	D 22-H 0	120	D 22-H 0	292	D 22-H 0	221	D 22-H 0	552
D 25-H 0	313	D 25-H 0	232	D 25-H 0	125	D 25-H 0	339	D 25-H 0	161	D 25-H 0	569
D 29-H 0	364	D 29-H 0	216	D 29-H 0	150	D 29-H 0	460	D 29-H 0	319	D 29-H 0	393
D 32-H 0	282	D 32-H 0	215	D 32-H 0	154	D 32-H 0	311	D 32-H 0	346	D 32-H 0	510
D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS	D 33-H 0	NS
D 36-H 0	227	D 36-H 0	287	D 36-H 0	NS	D 36-H 0	401	D 36-H 0	332	D 36-H 0	497
D 39-H 0	263	D 39-H 0	301	D 39-H 0	201	D 39-H 0	405	D 39-H 0	342	D 39-H 0	594
D 42-H 0	251	D 42-H 0	178	D 42-H 0	201	D 42-H 0	NS	D 42-H 0	182	D 42-H 0	335
D 42-H 0.5	147	D 42-H 0.5	236	D 42-H 0.5	166	D 42-H 0.5	NS	D 42-H 0.5	242	D 42-H 0.5	375
D 42-H 1	126	D 42-H 1	151	D 42-H 1	203	D 42-H 1	NS	D 42-H 1	375	D 42-H 1	344
D 42-H 2	227	D 42-H 2	403	D 42-H 2	319	D 42-H 2	NS	D 42-H 2	199	D 42-H 2	381
D 42-H 3	255	D 42-H 3	481	D 42-H 3	307	D 42-H 3	NS	D 42-H 3	514	D 42-H 3	524
D 42-H 4	139	D 42-H 4	324	D 42-H 4	390	D 42-H 4	NS	D 42-H 4	372	D 42-H 4	480
D 42-H 6	256	D 42-H 6	431	D 42-H 6	233	D 42-H 6	NS	D 42-H 6	163	D 42-H 6	383
D 42-H 8	181	D 42-H 8	326	D 42-H 8	230	D 42-H 8	NS	D 42-H 8	270	D 42-H 8	363
D 42-H 10	207	D 42-H 10	340	D 42-H 10	367	D 42-H 10	NS	D 42-H 10	284	D 42-H 10	484
D 42-H 12	217	D 42-H 12	314	D 42-H 12	251	D 42-H 12	NS	D 42-H 12	326	D 42-H 12	368
D 43-H 0	222	D 43-H 0	420	D 43-H 0	225	D 43-H 0	NS	D 43-H 0	406	D 43-H 0	377
D 44-H 0	205	D 44-H 0	255	D 44-H 0	204	D 44-H 0	NS	D 44-H 0	270	D 44-H 0	563
D 45-H 0	NS	D 45-H 0	NS	D 45-H 0	162	D 45-H 0	NS	D 45-H 0	272	D 45-H 0	NS
D 48-H 0	113	D 49-H 0	198	D 48-H 0	NS	D 48-H 0	NS	D 48-H 0	188	D 48-H 0	NS
D 51-H 0	73.5	D 51-H 0	168	D 51-H 0	NS	D 51-H 0	NS	D 51-H 0	106	D 51-H 0	NS
D 54-H 0	73.5	D 54-H 0	106	D 54-H 0	NS	D 54-H 0	NS	D 54-H 0	72.2	D 54-H 0	NS
D 57-H 0	42.2	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	NS	D 57-H 0	26.9	D 57-H 0	NS
D 72-H 0	22.0	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	NS	D 72-H 0	*	D 76-H 0	38.9
D 180-H 0	*	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	NS	D 180-H 0	*	D 180-H 0	*

17		18		19		20	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
D 1-H 0	*	D 1-H 0	*	D 1-H 0	*	D 1-H 0	*
D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*	D 1-H 0.5	*
D 1-H 1	*	D 1-H 1	*	D 1-H 1	*	D 1-H 1	*
D 1-H 2	*	D 1-H 2	*	D 1-H 2	*	D 1-H 2	*
D 1-H 3	*	D 1-H 3	22.6	D 1-H 3	*	D 1-H 3	18.2
D 1-H 4	*	D 1-H 4	22.6	D 1-H 4	*	D 1-H 4	24.6
D 1-H 6	*	D 1-H 6	23.3	D 1-H 6	17.2	D 1-H 6	29.4
		D 1-H 8	31.3	D 1-H 8	15.3	D 1-H 8	29.0
		D 1-H 10	32.7	D 1-H 10	15.3	D 1-H 10	30.3
		D 1-H 12	28.9	D 1-H 12	20.1	D 1-H 12	31.7
		D 2-H 0	95.7	D 2-H 0	16.4	D 2-H 0	48.7
		D 3-H 0	24.8	D 3-H 0	33.6	D 3-H 0	48.0
		D 4-H 0	124	D 4-H 0	40.2	D 4-H 0	105
		D 4-H 2	116	D 4-H 2	60.0	D 4-H 2	92.7
		D 4-H 4	138	D 4-H 4	62.2	D 4-H 4	106
		D 4-H 6	126	D 4-H 6	65.6	D 4-H 6	26.0
		D 4-H 8	91.6	D 4-H 8	77.0	D 4-H 8	139
		D 4-H 12	96.8	D 4-H 12	58.5	D 4-H 12	108
		D 5-H 0	135	D 5-H 0	66.9	D 5-H 0	113
		D 6-H 0	190	D 6-H 0	88.6	D 6-H 0	128
		D 7-H 0	182	D 7-H 0	99.5	D 7-H 0	147
		D 7-H 2	153	D 7-H 2	116	D 7-H 2	159
		D 7-H 4	213	D 7-H 4	115	D 7-H 4	201
		D 7-H 6	191	D 7-H 6	107	D 7-H 6	155
		D 7-H 8	207	D 7-H 8	49.5	D 7-H 8	159
		D 7-H 12	153	D 7-H 12	68.0	D 7-H 12	131
		D 8-H 0	204	D 8-H 0	120	D 8-H 0	169
		D 9-H 0	235	D 9-H 0	119	D 9-H 0	169
		D 10-H 0	235	D 10-H 0	157	D 10-H 0	184
		D 11-H 0	239	D 11-H 0	147	D 11-H 0	184
		D 12-H 0	299	D 12-H 0	187	D 12-H 0	183
		D 13-H 0	265	D 13-H 0	176	D 13-H 0	190
		D 14-H 0	267	D 14-H 0	252	D 14-H 0	183
		D 14-H 2	222	D 15-H 0	222	D 14-H 2	178
		D 14-H 4	293	D 15-H 2	242	D 14-H 4	176
		D 14-H 6	306	D 15-H 4	223	D 14-H 6	205
		D 14-H 8	267	D 15-H 6	151	D 14-H 8	209
		D 14-H 12	218	D 15-H 8	179	D 14-H 12	171

Final Data Hal/P 95-4 Chiral

5/4/98

17		18		19		20	
(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC	(hrs) Time	(ng/ml) CONC
		D 15-H 0	217	D 15-H 12	110	D 15-H 0	139
		D 16-H 0	211	D 16-H 0	186	D 16-H 0	172
		D 17-H 0	219	D 17-H 0	212	D 17-H 0	197
		D 18-H 0	216	D 18-H 0	265	D 18-H 0	190
		D 19-H 0	216	D 19-H 0	136	D 19-H 0	20.0
		D 20-H 0	219	D 20-H 0	127	D 20-H 0	162
		D 21-H 0	202	D 21-H 0	183	D 21-H 0	134
		D 21-H 2	254	D 21-H 2	138	D 21-H 2	176
		D 21-H 4	245	D 21-H 4	109	D 21-H 4	192
		D 21-H 6	227	D 21-H 6	248	D 21-H 6	163
		D 21-H 8	253	D 21-H 8	285	D 21-H 8	170
		D 21-H 12	211	D 21-H 12	166	D 21-H 12	154
		D 22-H 0	197	D 22-H 0	186	D 22-H 0	153
		D 25-H 0	212	D 25-H 0	188	D 25-H 0	115
		D 29-H 0	182	D 29-H 0	231	D 29-H 0	201
		D 32-H 0	173	D 32-H 0	217	D 30-H 0	244
		D 33-H 0	NS	D 33-H 0	278	D 32-H 0	329
		D 36-H 0	NS	D 36-H 0	231	D 33-H 0	264
		D 39-H 0	NS	D 39-H 0	309	D 35-H 0	420
		D 42-H 0	384	D 42-H 0	179	D 39-H 0	385
		D 42-H 0.5	389	D 42-H 0.5	200	D 42-H 0	304
		D 42-H 1	377	D 42-H 1	253	D 42-H 0.5	330
		D 42-H 2	438	D 42-H 2	259	D 42-H 1	389
		D 42-H 3	394	D 42-H 3	298	D 42-H 2	346
		D 42-H 4	393	D 42-H 4	278	D 42-H 3	424
		D 42-H 6	402	D 42-H 6	293	D 42-H 4	416
		D 42-H 8	352	D 42-H 8	271	D 42-H 6	386
		D 42-H 10	335	D 42-H 10	286	D 42-H 8	429
		D 42-H 12	353	D 42-H 12	268	D 42-H 10	383
		D 43-H 0	294	D 43-H 0	247	D 42-H 12	304
		D 44-H 0	NS	D 44-H 0	214	D 43-H 0	309
		D 45-H 0	273	D 45-H 0	236	D 44-H 0	283
		D 48-H 0	291	D 48-H 0	72.5	D 45-H 0	309
		D 52-H 0	215	D 51-H 0	97.6	D 48-H 0	159
		D 54-H 0	141	D 54-H 0	50.0	D 51-H 0	117
		D 57-H 0	73.2	D 57-H 0	28.0	D 60-H 0	51.9
		D 72-H 0	43.3	D 72-H 0	*	D 72-H 0	48.8
		D 180-H 0	NS	D 180-H 0	*	D 180-H 0	64

Analytical Data: Hal/MB 96-1

Sample ID		Halofantrine	Metabolite	Sample ID		Halofantrine	Metabolite
MN	Day-Time	(ng/ml)	(ng/ml)	MN	Day-Time	(ng/ml)	(ng/ml)
54	8/22-0807	*	*	72	8/22-0836	27.6	*
54	8/22-1010	146	*	72	8/22-1028	*	*
54	8/22-1215	47.4	*	72	8/22-1220	*	*
54	8/22-1406	60.6	*	72	8/22-1416	*	*
54	8/22-1608	61.2	*	72	8/22-1620	*	*
54	8/23-0815	21.1	*	72	8/23-0844	*	*
54	8/24-0818	*	*	72	8/24-0850	*	*
54	8/25-0824	11.0	*	72	8/25-0834	*	*
54	8/26-0842	10.7	*	72	8/26-0830	*	*
54	8/29-0827	*	11.6	72	8/29-0833	13.6	*
54	8/31-0812	*	*	72	8/31-0827	*	*
55	8/22-0812	*	*	73	8/22-0826	20.5	*
55	8/22-1015	43.8	*	73	8/22-1031	209	18.1
55	8/22-1208	77.5	*	73	8/22-1230	228	21.9
55	8/22-1410	95.8	13.8	73	8/22-1423	115	14.9
55	8/22-1612	47.9	*	73	8/22-1625	115	22.1
55	8/23-0819	12.8	*	73	8/23-0829	42.9	*
55	8/24-0822	12.7	*	73	8/24-0832	34.3	*
55	8/25-0826	10.7	*	73	8/25-0836	70.6	23.1
55	8/26-0846	*	*	73	8/26-0850	89.8	19.1
55	8/29-0830	11.0	29.6	73	8/29-0840	25.7	10.2
55	8/31-0810	*	*	73	8/31-0829	16.8	*
60	8/22-0816	*	*	74	8/22-0822	14.1	*
60	8/22-1021	114	13.6	74	8/22-1033	63.0	*
60	8/22-1217	189	25.2	74	8/22-1225	27.0	*
60	8/22-1414	88.6	20.5	74	8/22-1425	19.9	*
60	8/22-1615	91.9	19.9	74	8/22-1628	10.4	*
60	8/23-0823	52.0	*	74	8/23-0831	21.6	*
60	8/24-0826	15.7	*	74	8/24-0900	*	122
60	8/25-0828	99.0	17.8	74	8/25-0840	49.4	13.4
60	8/26-0847	42.4	*	74	8/26-0855	113	17.6
60	8/29-0832	245	35.3	74	8/29-0844	27.8	*
60	8/31-0815	22.9	*	74	8/31-0831	21.1	*
71	8/22-0840	28.2	*	75	8/22-0830	11.4	*
71	8/22-1025	*	*	75	8/22-1035	54.3	*
71	8/22-1223	*	*	75	8/22-1234	56.8	*
71	8/22-1420	*	*	75	8/22-1428	39.0	*
71	8/22-1618	*	*	75	8/22-1630	20.9	*
71	8/23-0839	*	*	75	8/23-0835	12.7	*
71	8/24-0846	*	*	75	8/24-0842	51.1	*
71	8/25-0832	14.6	*	75	8/25-0842	47.5	20.4
71	8/26-0826	*	*	75	8/26-0857	61.4	12.6
71	8/29-0835	13.4	*	75	8/29-0846	*	*
71	8/31-0825	*	*	75	8/31-0833	39.6	*

* Below Assay Sensitivity (10.0 ng/ml)

Analytical Data: Hal/MB 96-1

Sample ID		Halofantrine	Metabolite
MN	Day-Time	(ng/ml)	(ng/ml)
78	8/22-0858	*	*
78	8/22-1045	*	150
78	8/22-1245	*	171
78	8/22-1443	*	122
78	8/22-1640	*	54.5
78	8/23-0905	*	35.5
78	8/24-0914	*	82.9
78	8/25-0854	*	95.0
78	8/26-0908	31.9	110
78	8/29-0850	10.2	31.1
78	8/31-0845	*	18.1
79	8/22-0845	*	*
79	8/22-1043	*	*
79	8/22-1242	*	*
79	8/22-1437	*	*
79	8/22-1638	*	*
79	8/23-0850	*	*
79	8/24-0855	17.6	*
79	8/25-0852	*	32.4
79	8/26-0836	*	*
79	8/29-0847	*	68.0
79	8/31-0842	*	73.7
81	8/22-0901	*	25.7
81	8/22-1047	*	77.1
81	8/22-1249	*	57.6
81	8/22-1440	*	55.0
81	8/22-1645	*	43.6
81	8/23-0909	*	*
81	8/24-0912	18.9	148
81	8/25-0856	18.8	79.7
81	8/26-0912	*	25.3
81	8/29-0852	*	31.2
81	8/31-0844	*	31.8
82	8/22-0850	*	*
82	8/22-1040	*	46.6
82	8/22-1240	*	32.5
82	8/22-1433	*	24.2
82	8/22-1632	*	25.5
82	8/23-0853	*	20.8
82	8/24-0838	53.9	14.5
82	8/25-0845	*	117
82	8/26-0900	90.7	105
82	8/29-0812	*	297
82	8/31-0838	*	382
83	8/22-0854	*	*
83	8/22-1036	*	114
83	8/22-1237	*	174
83	8/22-1430	*	118
83	8/22-1635	*	59.8
83	8/23-0858	*	28.6
83	8/24-0904	11.9	143
83	8/25-0849	*	74.5
83	8/26-0904	12.7	89.1
83	8/29-0816	*	139
83	8/31-0835	*	115
84	8/22-0910	*	382
84	8/22-1053	*	19.2
84	8/22-1255	*	45.6
84	8/22-1446	*	49.2
84	8/22-1650	*	28.6
84	8/23-0912	*	17.4
84	8/24-0916	*	60.6
84	8/25-0859	*	37.6
84	8/26-0915	17.0	45.8
84	8/29-0820	*	76.5
84	8/31-0848	*	116
85	8/22-0907	*	24.1
85	8/22-1050	*	142
85	8/22-1253	*	206
85	8/22-1450	*	154
85	8/22-1647	*	99.0
85	8/23-0916	*	91.6
85	8/24-0925	*	108
85	8/25-0903	*	50.8
85	8/26-0922	*	35.9
85	8/29-0825	*	50.5
85	8/31-0850	*	229

* Below Assay Sensitivity (10.0 ng/ml)

Final WR 238605 Results

Sample No.	Sample ID	Plasma WR238605 (ng/ml)	Blood WR238606 (ng/ml)
1	22-B(6/08/15)	*	*
2	22-B(6/08/16)	443	603
3	22-B(6/08/17)	437	584
4	22-B(6/08/23)	828	1230
5	22-B(6/08/26)	1490	2310
6	22-B(6/09/13)	609	1130
7	22-B(6/09/27)	450	656
	23-C(6/08/16)	NS	*
2	23-C(6/08/17)	414	525
3	23-C(6/08/18)	405	688
1	24-A(6/08/20)	*	*
2	24-A(6/08/21)	252	307
3	24-A(6/08/22)	312	376
4	24-A(6/08/28)	1390	2320
5	24-A(6/09/04)	679	1230
6	24-A(6/09/18)	530	643
7	24-A(6/10/02)	395	435
8	24-A(6/10/16)	217	291
9	24-A(6/11/13)	78.7	80.6
10	24-A(6/12/11)	15.7	18.4
11	24-A(7/01/08)	5.56	4.27
12	24-A(7/02/05)	*	*
1	25-B(6/08/25AM)	2.24	*
2	25-B(6/08/25PM)	749	1120
3	25-B(6/08/26)	1260	1790
4	25-B(6/08/30)	1160	2040
5	25-B(6/09/08)	1380	2610
6	25-B(6/09/22)	867	1190
7	25-B(6/10/06)	571	712
8	25-B(6/10/10)	324	460
9	25-B(6/11/09)	192	238
10	25-B(6/12/15)	34	37.7
11	25-B(7/01/12)	6.01	8.10
12	25-B(7/02/09)	*	*
1	27-C(6/08/28)	*	*
2	27-C(6/08/29)	729	794
3	27-C(6/08/30)	578	922
4	27-C(6/09/03)	383	455

WR 238605 concentrations in ng/ml. * = below lower limit of quantitation (2 ng/ml in plasma and 4 ng/ml in blood). bc= chromatogram unacceptable, will be reanalyzed.

WR5/BP 96-2
Final WR 238605 Results

Sample No.	Sample ID	Plasma	Blood
		WR238605	WR238606
		(ng/ml)	(ng/ml)
5	27-C(6/09/08)	303	298
1	28-A(6/08/30)	*	*
1	30-A(6/08/30)	*	*
2	30-A(6/08/31)	423	429
3	30-A(6/09/01)	940	1150
1	32-C(6/09/03)	7.44	*
2	32-C(6/09/04)	391	412
3	32-C(6/09/05)	551	670
4	32-C(6/09/14)	356	411
5	32-C(6/09/18)	304	327
6	32-C(6/10/02)	175	190
7	32-C(6/10/16)	96.6	104
8	32-C(6/10/30)	54.6	54.8
9	32-C(6/11/27)	16.5	16.3
10	32-C(6/12/25)	6.21	5.22
1	34-B(6/09/09)	*	*
2	34-B(6/09/10)	649	608
3	34-B(6/09/11)	1520	1990
4	34-B(6/09/13)	1620	2100
5	34-B(6/09/20)	1690	2410
6	34-B(6/10/08)	667	1050
7	34-B(6/10/22)	530	646
1	35-C(6/09/10)	*	*
2	35-C(6/09/12)	327	318
3	35-C(6/09/14)	285	328
4	35-C(6/09/21)	175	212
5	35-C(6/09/25)	154	182
6	35-C(6/10/09)	90.3	112
7	35-C(6/10/24)	39.3	55.1
8	35-C(6/11/06)	24	30.0
9	35-C(6/12/04)	8.75	11.6

WR 238605 concentrations in ng/ml. * = below lower limit of quantitation (2 ng/ml in plasma and 4 ng/ml in blood). bc= chromatogram unacceptable, will be reanalyzed.

Sample No.	Sample ID	Plasma	Blood
		WR238605	WR238606
		(ng/ml)	(ng/ml)
10	35-C(7/01/02)	2.34	4.44
11	35-C(7/01/29)	*	*
12	35-C(7/02/26)	*	*
1	36-(6/09/16)	2.08	*
1	37-B(6/09/19)	*	*
2	37-B(6/09/21)	359	729
3	37-B(6/09/23)	1230	2240
4	37-B(6/09/28)	1030	1580
5	37-B(6/09/30)	1250	2210
1	39-A(6/09/21)	*	*
2	39-A(6/09/23)	278	301
3	39-A(6/09/25)	898	1330
4	39-A(6/10/02)	1070	1660
5	39-A(6/10/06)	877	1260
6	39-A(6/10/20)	473	737
7	39-A(6/11/03)	360	419
8	39-A(6/11/22)	159	181
9	39-A(6/12/15)	62.2	70.7
10	39-A(7/01/12)	18.7	20.2
11	39-A(7/02/09)	5.33	5.90
12	39-A(7/03/09)	*	*
1	40-A(6/09/21)	*	*
2	40-A(6/09/23)	198	227
1	41-B(6/09/21)	*	*
2	41-B(6/09/23)	563	1010
3	41-B(6/09/25)	1450	2800
4	41-B(6/09/30)	1180	2030
5	41-B(6/10/06)	1270	2690
6	41-B(6/10/20)	716	1260
1	43-C(6/09/23)	*	*
2	43-C(6/09/25)	396	731
3	43-C(6/09/27)	511	1010
4	43-C(6/09/29)	388	606
5	43-C(6/10/04)	329	369
6	43-C(6/10/22)	132	168
7	43-C(6/11/05)	64.1	69.8
8	43-C(6/11/19)	43.3	47.0
9	43-C(6/12/17)	10.4	11.0

WR 238605 concentrations in ng/ml. * = below lower limit of quantitation (2 ng/ml in plasma and 4 ng/ml in blood). bc= chromatogram unacceptable, will be reanalyzed.

Sample No.	Sample ID	Plasma	Blood
		WR238605	WR238606
		(ng/ml)	(ng/ml)
10	43-C(7/01/14)	2.38	*
11	43-C(7/02/11)	*	*
12	43-C(7/03/11)	*	bc
1	45-A(6/09/26)	7.14	*
2	45-A(6/09/28)	295	373
3	45-A(6/09/30)	816	1350
1	46-C(6/09/30)	*	*
2	46-C(6/10/02)	732	725
3	46-C(6/10/04)	601	738
4	46-C(6/10/08)	443	514
5	46-C(6/10/11)	334	367
6	46-C(6/10/29)	116	147
7	46-C(6/11/12)	58	68.9
8	46-C(6/11/26)	36.4	37.0
9	46-C(6/12/24)	7.68	8.19
10	46-C(7/01/21)	*	*
1	47-B(6/10/03)	*	*
2	47-B(6/10/05)	588	613
3	47-B(6/10/07)	1470	2250
4	47-B(6/10/11)	847	1630
5	47-B(6/10/18)	1030	1820
6	47-B(6/11/01)	807	1200
7	47-B(6/11/15)	523	657
8	47-B(6/11/29)	360	433
9	47-B(6/12/27)	144	188
10	47-B(7/01/24)	48	51.5
11	47-B(7/02/21)	8.5	12.9
12	47-B(7/03/21)	2.3	*
1	49-C(6/10/04)	*	*
2	49-C(6/10/05)	370	498
3	49-C(6/10/06)	432	523
4	49-C(6/10/12)	294	407

WR 238605 concentrations in ng/ml. * = below lower limit of quantitation (2 ng/ml in plasma and 4 ng/ml in blood). bc= chromatogram unacceptable, will be reanalyzed.

Sample No.	Sample ID	Plasma	Blood
		WR238605	WR238606
		(ng/ml)	(ng/ml)
5	49-C(6/10/19)	220	274
6	49-C(6/11/02)	113	149
7	49-C(6/11/16)	83.5	112
8	49-C(6/11/30)	57.8	81.0
9	49-C(6/12/27)	27.9	38.8
10	49-C(7/01/25)	8.87	12.4
11	49-C(7/02/22)	2.78	4.90
12	49-C(7/03/22)	*	*
1	51-C(6/10/10)	*	*
2	51-C(6/10/11)	470	460
3	51-C(6/10/12)	385	438
4	51-C(6/10/16)	367	371
5	51-C(6/10/21)	647	729
6	51-C(6/11/08)	246	314
7	51-C(6/11/22)	195	172
8	51-C(6/12/06)	70	89.5
9	51-C(7/01/03)	25.5	29.9
10	51-C(7/01/31)	6.71	6.39
11	51-C(7/02/28)	3.58	*
12	51-C(7/03/28)	*	*
1	52-A(6/10/10)	*	*
2	52-A(6/10/11)	277	404
3	52-A(6/10/12)	637	1170
4	52-A(6/10/16)	1240	1710
5	52-A(6/10/21)	759	1410
6	52-A(6/11/08)	454	607
7	52-A(6/11/24)	301	368
8	52-A(6/12/06)	192	236
9	52-A(7/01/03)	74.3	89.2
10	52-A(7/02/01)	21.2	28.7
11	52-A(7/03/01)	6.24	6.49
12	52-A(7/03/29)	*	*
1	53-A(6/10/12)	*	*
2	53-A(6/10/13)	278	360
3	53-A(6/10/14)	757	1020
4	53-A(6/10/23)	1200	1680
5	53-A(6/10/27)	978	1370
1	54-B(6/10/12)	*	*
2	54-B(6/10/14)	375	383
3	54-B(6/10/16)	1290	1600
4	54-B(6/10/21)	926	1570
5	54-B(6/10/23)	1690	2380
6	54-B(6/11/10)	608	1060
7	54-B(6/11/24)	578	660
8	54-B(6/12/08)	551	538

WR 238605 concentrations in ng/ml. * = below lower limit of quantitation (2 ng/ml in plasma and 4 ng/ml in blood). bc= chromatogram unacceptable, will be reanalyzed.

Sample No.	Sample ID	Plasma	Blood
		WR238605	WR238606
		(ng/ml)	(ng/ml)
9	54-B(7/01/05)	196	240
10	54-B(7/03/04)	29.7	32.3
11	54-B(7/03/30)	12.8	13.7
1	55-A(6/10/12)	*	*
2	55-A(6/10/14)	393	534
3	55-A(6/10/16)	789	1440
4	55-A(6/10/18)	1190	1860
5	55-A(6/10/23)	1060	1780
6	55-A(6/11/10)	535	646
7	55-A(6/11/26)	380	410
8	55-A(6/12/08)	270	283
9	55-A(7/01/05)	76.3	101
10	55-A(7/02/02)	17.5	26.1
1	56-A(6/10/13)	*	*
2	56-A(6/10/15)	374	392
3	56-A(6/10/17)	928	1310
4	56-A(6/10/21)	1510	2070
5	56-A(6/10/24)	1140	1620
1	57-A(6/10/14)	*	*
2	57-A(6/10/15)	456	474
3	57-A(6/10/16)	363	471
4	57-A(6/10/22)	1650	2350
5	57-A(6/10/29)	973	1640
6	57-A(6/11/12)	545	686
1	59-B(6/10/18)	*	*
2	59-B(6/10/19)	*	*
3	59-B(6/10/20)	*	*
4	59-B(6/10/26)	600	602
5	59-B(6/10/29)	1170	1700
6	59-B(6/11/16)	585	577
7	59-B(6/11/30)	464	493
8	59-B(6/12/14)	374	403
9	59-B(7/01/12)	129	136
10	59-B(7/02/07)	28.5	35.8
11	59-B(7/03/07)	9.04	10.0
12	59-B(7/04/04)	2.06	*
1	60-C(6/10/18)	*	*
2	60-C(6/10/19)	600	657
3	60-C(6/10/20)	681	627
4	60-C(6/10/26)	338	438
5	60-C(6/11/02)	292	296
6	60-C(6/11/16)	167	208
7	60-C(6/11/30)	117	116
8	60-C(6/12/14)	62.8	77.8
9	60-C(7/01/12)	17.8	21.9

WR 238605 concentrations in ng/ml. * = below lower limit of quantitation (2 ng/ml in plasma and 4 ng/ml in blood). bc= chromatogram unacceptable, will be reanalyzed.

Sample No.	Sample ID	Plasma	Blood
		WR238605	WR238606
		(ng/ml)	(ng/ml)
10	60-C(7/02/09)	4.18	6.13
11	60-C(7/03/09)	*	*
12	60-C(7/04/06)	*	*
1	64-A(6/11/08)	*	*
2	64-A(6/11/10)	328	387
3	64-A(6/11/12)	1060	1480
4	64-A(6/11/16)	1600	2270
5	64-A(6/11/19)	1160	1860
6	64-A(6/12/07)	544	689
7	64-A(6/12/22)	384	528
8	64-A(7/01/05)	297	364
9	64-A(7/02/02)	116	141
10	64-A(7/03/02)	38.2	54.4
11	64-A(7/03/30)	13.5	19.1
12	64-A(7/04/27)	4.8	6.33
1	65-A(6/11/09)	*	*
2	65-A(6/11/10)	536	537
3	65-A(6/11/11)	1200	1560
4	65-A(6/11/20)	1410	1940
5	65-A(6/11/24)	1180	1650
6	65-A(6/12/08)	743	774
7	65-A(6/12/22)	492	512
8	65-A(7/01/05)	297	336
9	65-A(7/02/02)	99.6	122
10	65-A(7/03/02)	31.9	35.7
11	65-A(7/04/30)	2.43	*
1	66-B(6/11/15)	*	*
2	66-B(6/11/16)	457	681
3	66-B(6/11/17)	1090	1670
4	66-B(6/11/19)	1460	2730
5	66-B(6/11/26)	1650	2870
6	66-B(6/12/14)	644	1100
7	66-B(6/12/28)	450	779
8	66-B(7/01/11)	308	354
1	67-B(6/11/18)	*	*
2	67-B(6/11/20)	585	555
3	67-B(6/11/22)	1160	1560
4	67-B(6/11/27)	1260	1690
5	67-B(6/12/03)	1060	1780
1	69-A(6/11/25)	*	*

WR 238605 concentrations in ng/ml. * = below lower limit of quantitation (2 ng/ml in plasma and 4 ng/ml in blood). bc= chromatogram unacceptable, will be reanalyzed.

Final WR 238605 Results

Sample No.	Sample ID	Plasma	Blood
		WR238605	WR238606
		(ng/ml)	(ng/ml)
2	69-A(6/11/27)	346	392
3	69-A(6/11/29)	848	1340
4	69-A(6/12/06)	1010	1910
5	69-A(6/12/10)	731	1260
6	69-A(6/12/24)	574	665
7	69-A(7/01/07)	360	436
8	69-A(7/01/21)	179	217
9	69-A(7/02/19)	52.7	64.6
10	69-A(7/03/18)	15.2	17.0
1	71-B(6/12/10)	3.22	*
2	71-B(6/12/12)	490	418
3	71-B(6/12/14)	1150	1300
4	71-B(6/12/18)	957	1330
5	71-B(6/12/25)	1260	1880
6	71-B(7/01/08)	752	767
7	71-B(7/01/22)	569	602
8	71-B(7/02/05)	492	475
9	71-B(7/03/05)	161	175
10	71-B(7/04/02)	66.4	51.6
1	74-A(6/12/22)	*	*
2	74-A(6/12/23)	347	418
3	74-A(6/12/24)	696	672
4	74-A(6/12/28)	1510	2390
5	74-A(7/01/02)	1190	2050

WR 238605 concentrations in ng/ml. * = below lower limit of quantitation (2 ng/ml in plasma and 4 ng/ml in blood). bc= chromatogram unacceptable, will be reanalyzed.

WR 238605 in Dog Plasma
Final Data

Dose Group- Animal ID- Week	(ng/ml)	Dose Group- Animal ID- Week	(ng/ml)	Dose Group- Animal ID- Week	(ng/ml)	Dose Group- Animal ID- Week	(ng/ml)
1M-09--1	*	2M-07--1	*	3M-10--1	*	4M-08--1	*
1M-09-4	*	2M-07-4	46.8	3M-10-4	353	4M-08-4	1590
1M-09-8	*	2M-07-8	54.5	3M-10-8	439	4M-08-8	1820
1M-09-13	*	2M-07-13	43.6	3M-10-13	382	4M-08-13	1820
1M-09-26	*	2M-07-26	52.1	3M-10-26	348	4M-08-26	1810
1M-09-40	*	2M-07-40	45.4	3M-10-40	429	4M-08-40	1500
1M-09-52	*	2M-07-52	73.1	3M-10-52	408	4M-08-52	1340
1M-11--1	*	2M-19--1	*	3M-13--1	*	4M-18--1	*
1M-11-4	*	2M-19-4	67.6	3M-13-4	182	4M-18-4	990
1M-11-8	*	2M-19-8	90.9	3M-13-8	212	4M-18-8	908
1M-11-13	*	2M-19-13	80.9	3M-13-13	246	4M-18-13	946
1M-11-26	*	2M-19-26	67.7	3M-13-26	146	4M-18-26	955
1M-11-40	*	2M-19-40	86.1	3M-13-40	149	4M-18-40	790
1M-11-52	*	2M-19-52	77.9	3M-13-52	168	4M-18-52	754
1M-15--1	*	2M-23--1	*	3M-14--1	*	4M-21--1	*
1M-15-4	*	2M-23-4	35.5	3M-14-4	360	4M-21-4	1380
1M-15-8	*	2M-23-8	37.8	3M-14-8	491	4M-21-8	1410
1M-15-13	*	2M-23-13	26.6	3M-14-13	564	4M-21-13	2240
1M-15-26	*	2M-23-26	28.3	3M-14-26	377	4M-21-26	1170
1M-15-40	*	2M-23-40	32.5	3M-14-40	514	4M-21-40	1350
1M-15-52	*	2M-23-52	30.6	3M-14-52	434	4M-21-52	1530
1M-22--1	*	2M-24--1	*	3M-17--1	*	4M-26--1	*
1M-22-4	*	2M-24-4	33.3	3M-17-4	428	4M-26-4	1520
1M-22-8	*	2M-24-8	42.1	3M-17-8	595	4M-26-8	1480
1M-22-13	*	2M-24-13	41.7	3M-17-13	532	4M-26-13	1750
1M-22-26	*	2M-24-26	36.9	3M-17-26	462	4M-26-26	1160
1M-22-40	*	2M-24-40	43	3M-17-40	471	4M-26-40	1370
1M-22-52	*	2M-24-52	46.1	3M-17-52	495	4M-26-52	1120

* = below assay sensitivity (4.00 ng/ml)

WR 238605 in Dog Plasma
Final Data

Dose Group-Animal ID-Week	(ng/ml)	Dose Group-Animal ID-Week	(ng/ml)	Dose Group-Animal ID-Week	(ng/ml)	Dose Group-Animal ID-Week	(ng/ml)
1F-29--1	*	2F-34--1	*	3F-28--1	*	4F-33--1	*
1F-29-4	*	2F-34-4	53.7	3F-28-4	183	4F-33-4	1540
1F-29-8	*	2F-34-8	57	3F-28-8	266	4F-33-8	1770
1F-29-13	*	2F-34-13	52.1	3F-28-13	338	4F-33-13	1470
1F-29-26	*	2F-34-26	74.6	3F-28-26	292	4F-33-26	1480
1F-29-40	*	2F-34-40	47	3F-28-40	241	4F-33-40	1690
1F-29-52	*	2F-34-52	61.8	3F-28-52	302	4F-33-52	1200
1F-30--1	*	2F-35--1	*	3F-31--1	*	4F-36--1	*
1F-30-4	*	2F-35-4	57.5	3F-31-4	405	4F-36-4	1390
1F-30-8	*	2F-35-8	78.7	3F-31-8	650	4F-36-8	1820
1F-30-13	*	2F-35-13	83.7	3F-31-13	604	4F-36-13	2390
1F-30-26	*	2F-35-26	148	3F-31-26	414	4F-36-26	1240
1F-30-40	*	2F-35-40	76.2	3F-31-40	432	4F-36-40	1520
1F-30-52	*	2F-35-52	83.7	3F-31-52	532	4F-36-52	1490
1F-38--1	*	2F-37--1	*	3F-40--1	*	4F-41--1	*
1F-38-4	*	2F-37-4	26.8	3F-40-4	221	4F-41-4	1240
1F-38-8	*	2F-37-8	28.9	3F-40-8	357	4F-41-8	1490
1F-38-13	*	2F-37-13	38.7	3F-40-13	249	4F-41-13	1600
1F-38-26	*	2F-37-26	27.7	3F-40-26	257	4F-41-26	1210
1F-38-40	*	2F-37-40	70.7	3F-40-40	247	4F-41-40	1390
1F-38-52	*	2F-37-52	32	3F-40-52	218	4F-41-52	1450
1F-42--1	*	2F-45--1	*	3F-43--1	*	4F-44--1	*
1F-42-4	*	2F-45-4	26.7	3F-43-4	326	4F-44-4	1680
1F-42-8	*	2F-45-8	40.8	3F-43-8	396	4F-44-8	1180
1F-42-13	*	2F-45-13	46.2	3F-43-13	367	4F-44-13	1610
1F-42-26	*	2F-45-26	41.3	3F-43-26	367	4F-44-26	1410
1F-42-40	*	2F-45-40	44.6	3F-43-40	444	4F-44-40	1440
1F-42-52	*	2F-45-52	51.9	3F-43-52	533	4F-44-52	2040

* = below assay sensitivity (4.00 ng/ml)

(hrs)	(ng/ml)
Time	CONC
22-48h(5/1/96)	2360
41-D0(5/30/96)	*
41-48h(6/1/96)	1320
42-D0(6/3/96)	*
42-48h(6/5/96)	1660
43-48h(6/9/96)	1950
44-48h(6/9/96)	1640
45a-D0(6/8/96)	22.0
45b-D0(6/8/96)	22.4
45-12h(6/9/96)	556
45-48h(6/10/96)	578
47-48h(6/13/96)	2070
50-48h(6/15/96)	1330
56-48h(6/23/96)	834
55-48h(6/20/96)	NS

* = below assay sensitivity: 8 ng/ml

Routine Analysis Mef/P 97-2
Results for Primaquine and Chloroquine and Metabolites

Sample ID	Primaquine (ng/ml)	Carboxy primaquine (ng/ml)	Chloroquine (ng/ml)	Monodesethyl Chloroquine (ng/ml)	Didesethyl Chloroquine (ng/ml)
005 Heparin	0	756	101	87.0	23.8
005 EDTA	0	722	147	172	43.1
LLOQ	28.5	50.0	20.0	20.0	20.0

For the chloroquine and monodesethyl chloroquine results, the controls exceeded acceptable limits (>50% of controls with >|±15%| error. Should we repeat these assays (limits would not be exceeded, if the rule was ">50% of controls with >|±22%| error"), or is this data sufficient for your needs?